

**ENVIRONMENTAL FACTORS INFLUENCING THE
BREEDING AND HEALTH OF A PREDATOR ENDEMIC TO
SOUTHERN AFRICA: THE ENDANGERED BLACK
HARRIER *CIRCUS MAURUS***



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**Thesis presented for the degree of Doctor of Philosophy
January 2017**

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Recommended citation:

García-Heras M.-S. 2017. Environmental factors influencing the breeding and health of a predator endemic to southern Africa: the Endangered Black Harrier *Circus maurus*. PhD thesis, FitzPatrick Institute of African Ornithology, University of Cape Town, South Africa.

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"If we knew what it was we were doing, it would not be called research, would it"

Albert Einstein

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Acknowledgements

There are so many people to thank! Those who were physically present, and others that, despite the geographical distances, have always been there supporting me in achieving this incredible goal. A sincere thanks to all of you.

Firstly, I am extremely thankful to Dr. Rob E. Simmons for answering the email I sent him back in April, 2012, when I was still looking for PhD opportunities. Rob gave me the incredible chance to come to South Africa in September 2012, when I started doing fieldwork within his Black Harrier research project. I still remember the first time I saw this majestic bird of prey flying over the flowery Fynbos vegetation at the Silverstroom study site... An adult male was carrying prey in its yellow tarsi when, suddenly, after listening to this typical sharp food call, a wonderful female appeared from nowhere, flying towards him. A few seconds later, an incredible food pass between both creatures took place, and at that moment I stayed there, completely amazed by the beauty of the spectacle that just happened before me. What an incredible way to start what would become this extraordinary journey of more than 4 years! Rob, you taught me almost everything I know now about Black Harriers, from how to find a nest in very dense vegetation and harsh conditions, to how read an individual's behaviour and interpret its signs - I have learned an incredible amount during these years. I am very thankful for that! I am also very grateful for having been able to use all the precious data you have been collecting since 2000 and for all of your hard work correcting drafts and improving my English! I am looking forward to our future collaborations.

To Beatriz Arroyo and François Mougeot - I have so many things to say, that I don't even know where to begin! What an outstanding Spanish-French couple! You taught me an incredible amount of things, from helping me conceive and implement diverse fieldwork technics and tricks, how to efficiently catch adult Black Harriers, how to analyse data and ask yourself the right questions, bettered my English in writing papers, how to bring confidence when giving a talk at a conference, and how to take a step back and cool down in difficult and challenging situations. Thank you so much for your hard work correcting drafts, for always being so available and responsive, for spending the many hours skyping between Cape Town and Ciudad Real, and for the stimulating chats we have had around a drink, talking in our favourite language, "Frenchspanglish". I am definitely looking forward to our future projects! I am also very grateful for letting me stay at your house when I was visiting Ciudad Real and for making me feel, since the very beginning, that I was part of your family. I also want to thank Gaelle and Yan, their two kids, who I have seen growing up and evolving over the last four years – thanks for the many fun moments and laughs!

To Prof. Graham Cumming, who was initially my official supervisor. Thank you for supporting my research at the very beginning and for the economic support you provided! Dr. Arjun Amar joined as my official supervisor in 2015, after Graham left the Fitz towards his new Australian adventures. Thank you for the constructive chats and for improving many pieces of my work! To Peter Ryan, thanks for the chats we had about pollutants and contaminants and for being such an amazing director. To Susie Cunningham, thank you for your kindness, for the many chats about life and research, for staying calm and diplomatic in tough situations with a positive attitude! I felt very privileged to have been your office mate for more than two years!

A big thank you to the Fitz support staff. What a privilege and a wonderful experience it has been to be able to spend four years at this incredible Institute and grow up, little by little, as a scientist! To my dear Anthea Links, thank you for the many precious moments we had talking about everyday life, for letting me use the printer and scanner every time I needed it, for always sorting me out with field equipment, car bookings, and funding when issues emerged. I will miss your kindness and our daily Afrikaans teaching class “goeie more”, “goeie middag”, “Hoe gaan dit” – I owe you lots of chocolates! To Hilary Buchanan, thank you for being so efficient and to always work things out when the sky looked grey - I will be always very appreciative for all of your advice and help! Thanks to Denise Scheepers and Tania Jansen for the fun chats while making coffee and for contributing in improving my Afrikaans! To Susan Mvungi, thank you so much for always helping me to find the books or papers I couldn't find – you always found a way! To Grandville Faulmann, thanks for helping me build some very useful tools for my fieldwork! And finally, a general thank you to Rob Little, Gonzalo Aguilar, Chris Tobler and Margaret Koopman for always helping me when I needed it, and for all your advice.

To the Fitzies students and postdocs – what an incredible and diverse bunch of people! Special thanks to Eléonore Hellard for the many hours spent talking about research, PhD and life. Thanks for your emotional support in tough situations and for the incredible moments we have spent together! The Fitz would surely have another colouration if you wouldn't have been there these last 3 years – “Tu m'es bien précieuse ma Léo!”. To my ex-office mate Alex Thomson and my current office mates, Antje Steinfurth, Margaux Rat, and Tanja van de Ven – thank you so much for the many PhD chats, in person or from the Kgalagadi via WhatsApp, for your support and for the fun moments we spent at and outside the office! To Chevonne Reynolds, Gareth Tate, Rowen van Eeden, Megan Murgatroyd, Petra Sumasgutner, Davide Gaglio, Dominic Henry, Dominic Rollinson, Kristine Maciejewski, Hayley Clements, Samuel Temidayo Osinubi, Diana Boloppo, Jordan Calder, Beckie Garbett, Alex Atkins – thank you for the fun moments at the tea room, at the Friday UCT-pub or at the raptor research retreat! Also special thanks for those of you who helped me in the field when I needed four hands, and a special mention to Davide Gaglio for sneaking into the field and taking beautiful pictures of Black Harriers – Grazie amico!

To the IREC people – thank you so much for being so open when I was in Ciudad Real, for the stimulating chats about research, for giving me a hand whenever I needed it and for all the fun moments spent around “unas tapas y birras” – special mention for Jesus Caro, Marisa Sicilia, and Jose-Luis Guzmán! Also, special thanks to Rafael Mateo's research group, and most especially to Pablo Camarero and John Jairo López Perea for your precious help in the lab! To Txuso García, thanks for conducting DNA-analyses; Txuso and Juancho Luque-Larena, thanks for flying all the way from Spain to South Africa and helping with my fieldwork catching Black Harriers and small mammals – lots of fun moments and laughs!

To our other collaborators, Graham and Margaret Avery, thank you so much for your help with the identification of small mammal's jawbones and teeth, and bird's bones and feathers! To Michelle Maritz, thanks for analysing the blood smears; and to Nicholas Lindenberg and Thomas Slingsby thanks for helping with the GIS maps! To the field assistants, reserve and park managers, and sponsors – a big thank you for easing the access to the study areas, and making my fieldwork and research possible! Special thanks to Bettie Bester and Binks Mackenzie who have spent many

hours with me at the West Coast National Park during my first two breeding seasons – it has been a real pleasure! Also thanks to Marlei Martins for help in the field during my first breeding season. To François Van der Merwe, Steyn Marais, Pierre Nel, Andrew Jenkins, Odette Curtis, Anne Williams, and to BirdLife South Africa, Tygerberg bird club and especially Brian Sanderwalt, Inkwazi and Wits Bird Club, the Two Oceans Slope Soarers (TOSS), Natural Research UK, Hawk Mountain (USA), the Peregrine Fund, Sven Carlsson-Smith, Nial Perrins, Chris Cory, Gisela Ortner, and James Smith for your help and economical support! Finally, I also have to thank our stuffed spotted Eagle Owl, “Manolito” – without you buddy, we wouldn’t have been able to catch as many adult Black Harriers as we did, thank you for your great and dedicated work!

I also would like to thank all my frisbee friends, who really became my Cape Town family over the months and years – there are many of you, so I won’t write all the names here, but you all know who you are... I was extremely lucky to have met the right persons at the right moment when I first arrived to Cape Town, and without whom my life right now would be formed by a totally different pallet of colours. Christine Moore, you were key in this process! Thanks for introducing me to the ultimate Frisbee community, a sport that I ignored before moving here, but that right now I couldn’t imagine it to be outside of my life! I thank all my Catch 22 team-mates for the many moment and laughs spent together, in and outside the field, and for being so supportive and comprehensive when I had to choose my computer over throwing discs – “we all like the pew pew *pew pew*”! Special thanks to Brianna Mersey, Sandra Jordaan, Jonathan Aronson, Anna Coussens, Taryn Morris, Sid Rupani and Neal Lazarus – thanks for being amazing and for all the special moments we shared together over the last four years, for the many laughs while sipping a beer and watching sunsets a Clifton beach – I am looking very forward to many other moments like these! Thanks to Lindsay Demes and Paolo Denti for being the best flatmates one can possibly wish for! Thank you guys for your patience and understanding when I used our living room to dry the many Black Harrier pellets I collected in the field, or when I was analysing the many video footages and couldn’t stop talking about how amazing “my nestlings” were! Thanks for the many moments we spent at our “Happy House” – you guys made this adventure a lot funnier!

To all my friends around the world - thank you for being present and supportive despite the distance! Sometimes, it is tough to keep having a real relationship when you only see each other a couple of days a year, but it happened to be pretty easy with all of you – special thanks to my “Mainzeros - #PorFinOcurrió”, “Chicas”, “Pentanome” and “Atukus”!

To my French and Spanish family, my mother and my godfather Pepillo – thanks to all of you for your support, calls and cheerful messages since I have started this adventure. Being far hasn’t been always easy, and many times I would have wished so much to have been able to be physically closer, especially being by your side Irene García-Heras Carretero. Since I was little, you have always been a source of inspiration to me, and your dedication to your study and professional career have definitely contributed to push me over my limits and to achieve my dreams. Gracias por todo prima, estas muy presente en mi corazón, siempre. ¡Te quiero mi niña!

To my dad Jose-Luis and sister Anne-Laure – I couldn’t possibly use all of the words in the dictionary to express how lucky and proud I feel to have you in my life. You two are a continuous source of inspiration! Thank you for listening, at times for hours and hours, about my fieldwork, new experiences, my findings and how wonderful “my harriers” are! You can’t even imagine how much of

a relief it has been to be able to have you on the other side of the phone every time I needed it or was feeling down - you were always there for me, at any time, at any moment, no matter the circumstances, and always cheering me up and finding the adequate words for it. “¡Vuela águila, vuela lo más alto posible!” me decías papa... Thanks for always believing in me and for giving me the opportunity to follow my dreams. Si he logrado llegar hasta donde estoy hoy en día, desde luego es gracias a ti papa. Gracias a los dos por vuestra paciencia, ayuda, vuestros miles de consejos, y simplemente por ser como sois - no cambiéis nunca ¡Os quiero con todo mi corazón!

And finally, thank you to a very special person - Alan Hoyt. Thank you for believing in me since I started this project and for always doing your best to be there despite the 7-8 hour time difference, the 15 000 km and the ocean between us! Thanks for all these new experiences and travels we have experienced together, for making me discover the other side of your country which seemed so hostile to me before meeting you, and for boosting my English the way you did! You have spent hours listening to my presentations when I was training to give a talk for conferences, giving me advice on how to correctly pronounce some words, checking out articles and emails scanning for English mistakes, listening to my excitement about my new findings. Thank you for cheering me up the way you did and always finding the good words when I felt so stressed. Thank you for your support, advice, help, patience and kindness – I am looking very forward for the next adventures building memories with you by my side. I love you with all my heart.

Thesis Abstract

A general and increasing biodiversity loss has been observed since the 20th century. Faced with the extreme rapidity of population declines, conservation biologists seek to understand the limiting and regulating factors driving changes in animal populations. This is particularly important for rare species as small population size increases extinction risk. Birds are amongst the most studied animals in this context. As a group that occupies a high trophic level, raptors are particularly vulnerable to external changes and are generally regarded as useful indicators of ecological change.

The Black Harrier *Circus maurus* is an avian predator endemic to southern Africa, which breeds essentially along the South African coast within the Fynbos biome, and inland within the Karoo biome. Its population size has been estimated at less than 1,000 breeding birds, and the species is currently considered as Endangered in South Africa, Namibia and Lesotho. Although some studies have been conducted on Black Harriers in the last four decades, the reasons for its scarcity currently remain little known and insufficiently explored. Filling this knowledge gap is therefore essential for its conservation. In this context, the main goal of this thesis is to develop an overall comprehension of how various environmental factors may affect the breeding and health of this Endangered species, at both population and individual levels. I conducted my fieldwork during the 2012-2015 breeding seasons in two contrasting geographical regions: one along the west coast in the Western Cape Province, and the second one inland in the surroundings of Nieuwoudtville in the Northern Cape Province. For some chapters (Chapters 1-3), I analysed historical data collected by Dr. R. E. Simmons during 2000-2011 breeding seasons.

The Chapters 1-3 focus on investigating, at the population level, the spatial-temporal variations of the breeding phenology and success, and the diet composition, which are key parameters known to strongly influence the dynamics of wild animal species. I also look at the inter-relationship between those parameters. Using data from ca. 400 breeding events collected from 2000-2014 breeding seasons, I first investigate (Chapter 1) how key breeding parameters, i.e. clutch size, breeding success and productivity, varied with the timing of breeding, weather conditions (i.e. rainfall and temperatures) and between regions. I found an extended laying period of 8-months and a marked seasonal decline in all breeding parameters, that were more pronounced in inland than in coastal regions for clutch size and productivity. These, in turn were concomitant with weather conditions becoming drier and hotter in both regions as the breeding season progressed. These adverse conditions, however, were achieved more quickly in inland than in coastal regions. Clutches and productivity were overall greater, and laying occurred earlier under wetter conditions, but

temperatures were not found to have any direct influence on the timing of breeding or on any breeding performance parameters. However, warmer temperatures modulated Black Harriers' food availability and provisioning rate (see Chapter 3). These results suggest that optimal breeding conditions are more temporally limited inland than along the coast, and may explain why inland regions are not so frequently occupied by breeding Black Harriers in contrast to coastal regions.

In my next chapter (Chapter 2), I describe the diet composition of Black Harriers, using ca. 1,000 pellets (> 1,700 identified prey) collected at nest sites in the two geographical regions (coastal vs. inland), and over 10 breeding seasons (2006-2015). I confirm that the species is a small mammal specialist (64.4 % and 78.2 % of prey and consumed biomass, respectively), with the Four-Striped Mouse *Rhabdomys pumilio* as its main trophic resource. I also reveal the importance of birds and reptiles as alternative prey, particularly in the inland region, and showed inter-annual variations in diet in both regions.

Given that Black Harrier are specialist predators of small mammals, and that they showed seasonal declines in productivity, I further investigated in my Chapter 3 the spatial and temporal variations in the diet composition of breeding Black Harriers, and its relation with factors potentially affecting prey abundance (e.g. winter rainfall, which potentially affects primary production) or accessibility (e.g. daily temperatures which potentially affects prey behaviour). I found that the diet composition hardly changes throughout the breeding season in the coastal region; by contrast, I show a marked seasonal decline in the occurrence of small mammal prey in inland regions. Furthermore, using camera recordings set at nests during the 2014 breeding season to investigate daily patterns of prey provisioning rates, I show that small mammals were delivered markedly less often during the middle of the day at inland nests, whereas alternative prey were still delivered at a similar (reptiles) or slightly lower (birds) rate at this time of the day. I also found that the occurrence of small mammals in Black Harrier's diet significantly decreased with increasing temperatures, which supports the idea that hotter temperatures reduce small mammals' availability in inland regions relative to the coastal regions. All these results suggest that the steeper seasonal declines in clutch size and productivity observed in Black Harriers nesting inland (Chapter 1) are likely due to a lower primary prey availability there as the breeding season progresses, forcing breeders to consume harder to catch and/or less optimal prey for the species, such as birds or reptiles.

In Chapters 4 and 5, I use an eco-toxicological and eco-physiological approach to assess environmental contamination and its potential effects on wild individuals. Persistent pollutants such as Organochlorine Compounds (OCs) have been highlighted as a cause of population decline in apex predators. Understanding the patterns and sources of OC contamination, and its potential effects on

individual condition can be crucial for the conservation of affected species. In Chapter 4, I report on the occurrence of OCs in wild Black Harriers (90 nestlings, 15 adult females and 9 adult males), using blood samples collected during the 2012-2014 breeding seasons. Levels of polychlorinated biphenyls (Σ PCB) and dichlorodiphenyltrichloroethane (Σ DDT, for the sum of p,p'-DDT and its metabolite p,p'-DDE) were detected in 79 % and 84 % of sampled individuals, respectively. Nestlings had significantly more p,p'-DDT than adults, which may suggest a current and illegal use of DDT within the Black Harrier breeding range. Nestlings also had greater Σ PCB levels than adults, which increased with the density of electric transformers within the breeding territory, i.e. a suspected source of PCB contamination. The "Transformer Density Index" that I used, which took into account both number and kVA-rating power of those transformers per km², may represent a useful tool to assess the exposure of PCBs and the potential contamination to terrestrial wildlife in general. Levels of p,p'-DDE were greater in adults than in nestlings, and significantly increased with the percentage of wetlands within the breeding territory indicating the important role played by wetlands as source of contamination. Additionally, inland breeders who consume more birds, and especially farmland birds such as the Common Quail *Coturnix coturnix* (Chapter 2), also presented higher levels of p,p'-DDE in their blood. Furthermore, the OC levels appeared to induce sub-lethal effects, as apparent from associations with indicators of physiological condition. PCB-contaminated Black Harriers had increased heterophils to lymphocytes ratio, suggesting that they were physiologically stressed and immuno-suppressed. p,p'-DDT contaminated individuals had greater number of white blood cells.

For my last chapter (Chapter 5), I investigated associations between PCBs and DDTs blood-levels, circulating carotenoids and the yellow-orange colouration of integuments of Black Harrier nestlings. Their cere and tarsi are pigmented by carotenoids and may function as signals of healthiness, condition or need in a context of parent-offspring communication or sibling competition. A disruption of these coloured traits by contaminants may therefore have consequences for nestlings. I show a strong association between the orangeness-purity of the of cere and tarsi colouration and the levels of circulating carotenoids, confirming that higher levels of circulating carotenoids are associated with a more intense yellow-orange colouration. Both the colouration (i.e. orangeness-purity) and circulating carotenoid levels increased with nestling's age, and with an increasing proportion of bird biomass in the diet. These results suggest that the quality of the ingested food, i.e. in terms of carotenoid content, which is greater in bird than in small mammal prey, was a main determinant of carotenoid availability. There was no association between body condition and carotenoids or colouration suggesting food quantity per se was less important than diet composition to explain carotenoid intake. While consuming more birds seems beneficial for nestling Black Harriers as it increased the levels of circulating carotenoids, it also increased the

likelihood of ingesting DDT, which in turn also tended to worsen physiological condition (Chapter 4). Additionally, in chapter 5, I found that nestlings with more DDT had also lower levels of circulating carotenoids and a reduced carotenoid-based coloration (i.e. a yellow rather than orange and a less pure colouration). Circulating carotenoid levels and the orangeness-purity of coloured integuments were unrelated to blood PCB levels, although the brightness of integuments (i.e. lack of pigmentation) increased with PCB levels. Together, my results are consistent with the hypothesis that OC contaminants, in particular DDT, may disrupt carotenoid-based signaling in exposed nestlings.

The results of this thesis highlight the importance of multifaceted studies, at both the population and individual levels, when attempting to understand a species' limiting factors. I show the importance of the coastal region (Fynbos biome) for the stability and sustainability of the overall Black Harrier population. This region and the Fynbos habitat seems to provide better conditions for successful breeding in terms of food availability and weather conditions, but the habitat is also the most limited in space. Climate change may also dramatically influence Black Harrier's breeding phenology and breeding outputs, with likely changes in the availability of the primary prey (small mammals), forcing individuals to consume harder-to-catch and less optimal prey such as birds, which in turn also increases the likelihood of DDT contamination. Additionally, high densities of electricity transformers, which are found in more urbanized areas, increase the likelihood of PCB contamination. In turn, these OCs may have sub-lethal effects on contaminated individuals (e.g. affecting H:L-ratio, WBC count, or circulating carotenoid levels), and may disrupt the colouration of their integuments (i.e. cere and tarsi). Overall, the scarcity of Black Harriers may thus be related to a lack of optimal, i.e. unurbanised, unpolluted, unfragmented and food-rich, areas for breeding. The preservation and protection of the Fynbos should therefore be prioritized to insure optimal and sustainable conservation of Black Harriers in the long term, but also for the conservation of many other terrestrial species that face similar threats.

General Introduction



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The 20th and 21st centuries are marked by an increasing global biodiversity loss, directly linked with anthropogenic activities that are altering habitats and ecosystems on which species depend (Leakey & Lewin 1995; Butchart et al. 2010; Hoffmann et al. 2010). It has been estimated that one-fifth of the world's vertebrates are threatened with extinction (Hoffmann et al. 2010). Among this group, birds are particularly at risk with one in eight of the world's species being considered as globally threatened (BirdLife International 2013). Faced with the extreme rapidity of population declines, conservation biologists seek to understand the reasons behind variations in animal population abundance, and to identify the limiting factors of wild populations (Lack 1954; Soulé 1985; Simberloff 1988; Sutherland 1998; Newton 1998; Pimm et al. 2014). This central issue in ecology is essential for an effective and sustainable conservation of target species in space and time (Nilsson 1987; Newton 1998).

The external limiting factors known to affect wild populations dynamics include the access to resources, such as good-quality habitat and food (Martin 1995; Robinson et al. 1995; Sillett et al. 2000), competitors (i.e. inter- and intraspecific competition), and natural enemies such as predators, pathogens or parasites (Newton 1998; Begon et al. 2005). However, anthropogenic transformations of natural systems, such as habitat fragmentation, increase of urbanisation, overexploitation of resources, introduction of exotic species, and effects of climate change are expanding in many parts of the world, and play in many cases a more important role than natural extrinsic factors. For instance, changes in land use and habitat degradation may result in substantial landscape modifications, which consequently may induce dramatic impacts on wildlife (Saunders et al. 1991; Harrison & Bruna 1999). With habitat fragmentation, species become increasingly patchily distributed, reducing connections between different populations that in turn can lower the overall population size and viability (Newton 1998).

Contamination by anthropogenic pollutants have in particular been considered as a critical factor influencing wildlife in recent decades (Arroyo et al. 2016). Persistent organic pollutants (POPs) such as organochlorine pesticides (DDTs and its metabolites DDEs), and industrial products such as polychlorinated biphenyls (PCBs) have been highlighted as a cause of population decline in apex predators. Their high persistence and slow degradation

in the environment, and their ability for long-distance transportation and dispersion (Hoffman et al. 2003; Bustnes et al. 2004; Roscales et al. 2016) have facilitated their presence in all environmental ecosystems, from aquatic to terrestrial (Newton 1998; Hoffman et al. 2003). POPs are known to be extremely toxic to wildlife causing detrimental effects on the reproduction of several bird species (including embryo toxicity, eggshell thinning and breakage in the case of the effects of DDT in seabird and raptor species; e.g. Ratcliffe 1970; Newton & Haas 1988; Newton et al. 1986; Mateo et al. 2000; Wiesmuller et al. 2002; Cade & Burnham 2003;), as well as causing adverse effects on bird health, as evidenced using a wide range of physiological indicators (Naso et al. 2003; Bustnes et al. 2004; Bourgeon et al. 2012; Gómez-Ramírez et al. 2014; Ortiz-Santaliestra et al. 2015). In this context, it seems crucial to identify potential sources of contamination by POPs and investigate the effects that they may be inducing on wildlife (Newton, 1998).

Additionally, individual traits such as body condition, health status, individual foraging or mating abilities, or capacity to cope with pathogens or pollutants, are also known to play key roles in individual fitness (Gaston 1994; Terraube et al. 2014; Mougeot et al. 2016). Understanding how these parameters are linked together and whether they influence populations' dynamics through effects on survival or breeding performance may be thus highly relevant for the conservation of target species, especially those with a small population size and/or those that are sparsely distributed (see below). For instance, POPs can impair immune function (Grasman et al. 1996; Bustnes et al. 2004) and induce oxidative stress (Wayland et al. 2010; Ortiz-Santaliestra et al. 2015), which makes birds more susceptible to pathogens and parasites (Grasman et al. 1996; Grasman 2002). Other studies have also suggested that OCs could induce disruptive effects on the circulating carotenoid levels and the colouration of integuments in bird species (McCarty & Secord 2000; Bortolotti et al. 2003; Blévin et al. 2014). This can induce important negative effects on an individual's health, as circulating carotenoids serve important health related physiological functions (e.g. boosting immune response or antioxidant defences). Assessing these relationships at the individual level and quantifying the effects on certain traits may function as early warnings for progressing ecological effects, which may result in population declines (Peakall 1994; Handy et al. 2003).

All the above factors may contribute to reducing population sizes, increasing the rate of species loss and their possible extinction at a larger scale (Newton 1998; Real et al. 2010; Carvalho et al. 2011; Sutherland et al. 2011). Small populations and rare species are particularly at risk in this regard (Gaston 1994; Ryan & Siegfried 1994). Species are generally considered to be rare if they are uncommon, scarce or infrequently encountered, if their geographical ranges are limited, and/or if their abundances are low (Rabinowitz 1981; Rabinowitz et al. 1986; Prendergast et al. 1993; Gaston 1994; Yu & Dobson 2000). According to Stanley (1986), the size of a population is the primary factor that determines its vulnerability: species that exist in low numbers are usually more exposed to the loss of genetic diversity and can suffer from a diminished capacity to respond to environmental changes, such as pollutant contamination or climate change (e.g. increase of temperatures and decrease in rainfall, which may also affect primary prey abundance), and hence a reduced adaptive potential (Charlesworth & Charlesworth 1987; Swindell & Bouzat 2005). In bird species, for example it has been suggested that a population size of approximately 5000 individuals is necessary in order to survive environmental stochasticity, and that smaller populations are more exposed to genetic or demographic stochastic events (Soulé 1987; Gaston 1994). Therefore, species that are only distributed as small populations are always considered more vulnerable and of conservation concern, as they may not be able to recover from the latter stochastic events (Shaffer 1981, 1987; World Conservation Monitoring Center 1992; Ryan & Siegfried 1994).

Birds are amongst the most studied animals in this context. As with many species occupying a high trophic level, raptors are particularly vulnerable to external changes and are generally regarded as useful indicators of ecological changes and environmental health (Newton 1979, 1998, 2003). Among raptors, harrier species (*Circus spp*) are distributed around the world, and are present in different ecosystems with interspecific differences in terms of their biology and ecology (Simmons 2000). Some harrier species have been very well studied in Europe, such as the Montagu's Harrier *Circus pygargus*, Hen Harrier *Circus cyaneus* and European Marsh Harrier *Circus aeruginosus*; others remain very poorly studied (e.g. Madagascar *Circus macroscelus*, Reunion *Circus maillardi*, South American *Circus cinereus*, and Black Harriers *Circus maurus*) (see e.g. Simmons 2000). Populations among harrier species can be very variable, some species presenting a large population size such as

the Montagu's Harrier (> 40,000 mature individuals in Europe; Birdlife international 2012), while others are very scarce, such as the Black Harrier in southern Africa (< 1,000 mature individuals Simmons et al. 2005; Birdlife International 2013; Taylor 2015). The reasons for the scarcity or decline of a species are unlikely to be related to a single factor, but rather the result of a succession and additive effects of several factors (Gaston 1994). Although it is often impossible to demonstrate the causal roles of specific factors in the dynamics of a wild species (as this would require experimental manipulations), in some cases the reasons for a species' scarcity or decline have been identified either experimentally or through empirical observations, and actions to improve the conservation status of the species have been designed (e.g. California Condor *Gymnogyps californianus*: Mertz 1971; Wilbur 1978; Hen Harriers: Amar & Redpath 2002; Amar & Redpath 2005; Cheetah *Acinonyx jubatus*: Laurenson 1995; Harlequin frogs *Atelopus* sp.: Pounds & Crump 1994; la Marca et al. 2005; reptiles: Gibbons et al. 2000). Understanding how abiotic and biotic parameters may affect wild populations and use this information to better understand the potential factors playing a role in the scarcity of a species is important in the efficient design of sustainable conservation measures for those species (Sutherland et al. 2004).

OUTLINE OF THE THESIS

Within the latter context, the main goal of my PhD thesis is to develop an overall understanding of how various environmental factors may affect the breeding and health (i.e. physical and physiological condition) of the Endangered and scarce Black Harrier, at both the population and the individual level. Published information about this species at the beginning of this PhD was limited, comprising a few formal studies on the biology (Van der Merwe 1981), conservation status (Siegfried 1992), ecology (Simmons et al. 1998, 2005), habitat requirements (Curtis et al. 2004; Curtis 2005), genetics (Fuchs et al. 2014) and use as an indicator species (Jenkins et al. 2013). However, the reasons for its scarcity currently remain little known and insufficiently explored, and filling this knowledge gap is essential for its conservation. Below I introduce the specific aims of my PhD research, but I outline first some aspects of the ecology of Black Harriers, and describe some of the previous research conducted on the species during the last few decades.

THE BLACK HARRIER AS A STUDY SPECIES

Black Harrier ecology

The Black Harrier is a ground-nesting medium-sized bird of prey, endemic to southern Africa. The species has the most restricted distribution range of any continental harrier species, covering approximately 500,000 km², but with a far more restricted breeding range of approximately 170,000 km², located in south western South Africa (Van der Merwe 1981; Siegfried 1992; Simmons 2000) (Figure 1).

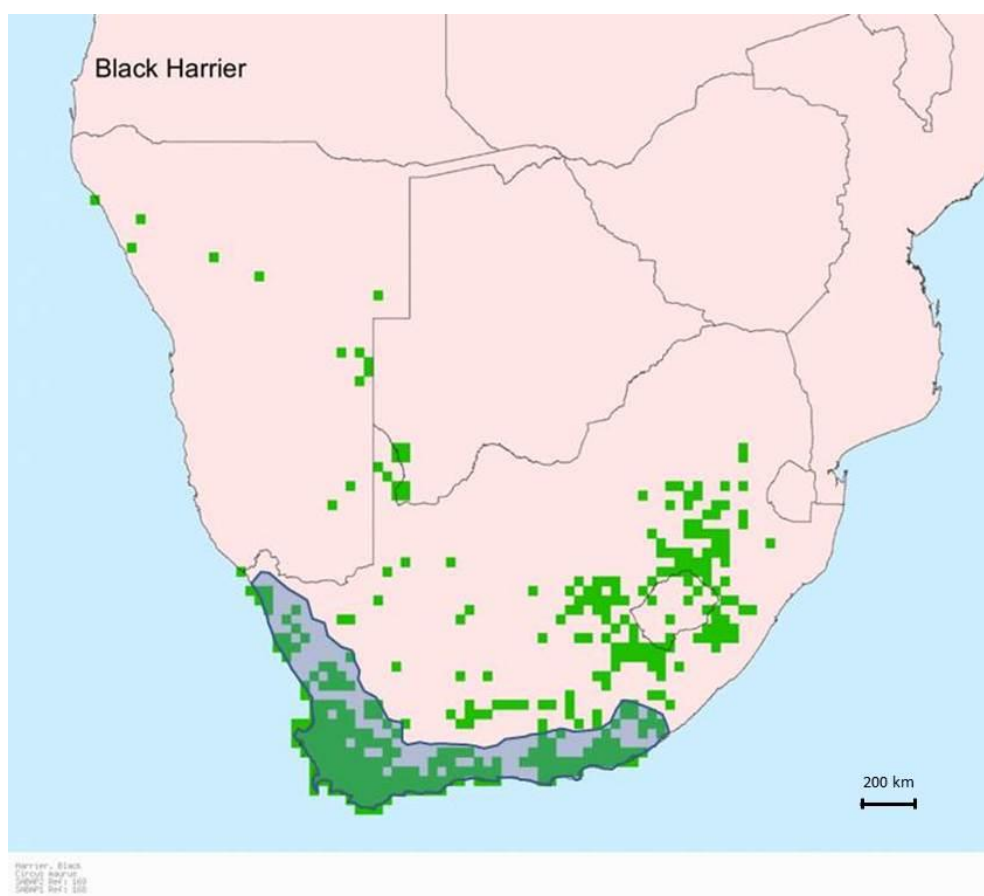


Figure 1. Global distribution of the Endangered Black Harrier *Circus maurus* within southern Africa. The breeding range is highlighted with the blue shaded area, which was added to the initial map downloaded from the South African Bird Atlas Project (SABAP) 2 data base (http://sabap2.adu.org.za/species_info.php?spp=169§ion=4#menu_left), for which permission was requested.

Black Harrier population size has been estimated at less than 1,000 breeding individuals, although it is impossible to determine the accuracy of this estimate. There is

currently not enough information or systematic censuses to provide another estimate. The species is currently considered as Endangered in South Africa, Namibia and Lesotho (Simmons et al. 2015; Taylor 2015). In official IUCN ranking, Black Harriers however remains as a “globally Vulnerable” species (BirdLife South Africa 2013), but this requires revision considering the re-classification in the later countries. It is unclear whether the Black Harrier population has overall decreased, increased or remained stable in recent decades essentially due to a lack of precise information about the historical distribution and the current spatial-abundance of the species within its breeding range. Nevertheless, comparisons of Black Harrier sighting occurrences and rates from the South African Bird Atlas project 1 (1987-1991) and 2 (2007 to present) suggest that the population may have declined over the last 4 decades (Figure 2) (Underhill 2016; Underhill & Brooks 2016a, b).

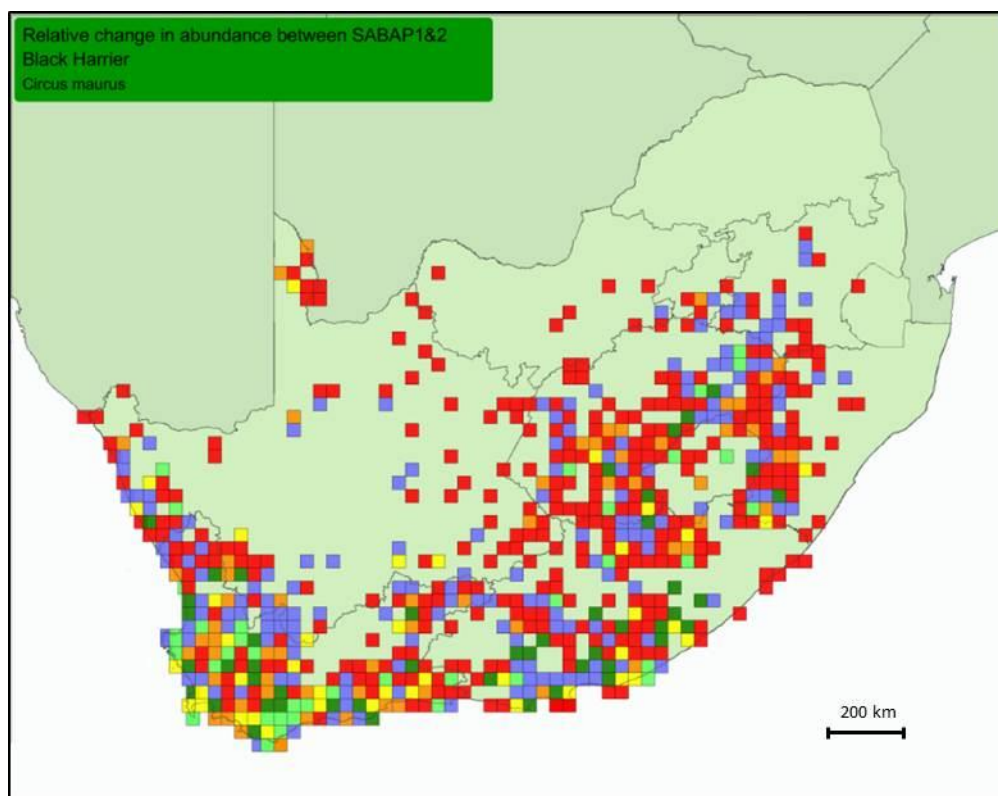


Figure 2. Range change map between SABAP 1 and SABAP 2 for the Black Harrier in South Africa Lesotho and Swaziland. The unit of data collection is the pentad or grid, five minutes of latitude by five minutes of longitude. Red, orange and yellow represent quarter degree grid cells with large, moderate and small relative decreases, while blue, dark green and light green represent grid cells with large, moderate and small increases. This map was downloaded from the South African Bird Atlas Project 2 data base, available online (<http://bo.adu.org.za/content.php?id=255>), for which permission was requested.

Unlike other raptors, Black Harrier breeders rarely re-use the same nest over the years, and it is as yet unclear if the same individuals breed together as a pair year after year, although evidence from ringing and satellite tagging suggests this is not the case (Simmons et al. 2005; RES and MSGH *personal observations*). The species is considered as mainly socially monogamous, but some cases of polygyny (two females per male) have been recorded (Simmons et al. 2005). At the beginning of the breeding season male Black Harriers attempt to attract a mate, by performing a spectacular flying display called "Skydancing"; a series of "U"-shaped undulations while calling at top of displays, as most harrier species do (Hamerstrom 1986; Simmons 2000; Simmons et al. 2005; Arroyo et al. 2013). During this courtship period, and before the laying starts (i.e. clutches can be initiated from mid-May to mid-December), Black Harrier males feed females; a behaviour that has been suggested to be important by providing extra nourishment at the time of egg formation in other species (Watson 1977). Both, males and females contribute to building the nest, even though females undertake the majority of it (Simmons 2000; Simmons et al. 2005). The Black Harrier's nest is a small structure of grasses, stems and small twigs of about 350-450 mm of diameter. It is usually placed on or just above ground, and often in rank marsh grasses or near Fynbos bushes or sedges (*Juncus spp*) (Simmons et al. 2005). The vegetation height around the nest is an average of 64 ± 32 cm [minimum-maximum range: 20 – 170 cm], and with low visibility to reduce terrestrial and aerial predation (Simmons et al. 2005; *unpublished data*). Eggs are usually laid at intervals of 48 hours, the incubation generally starts with the second-laid egg and last 31 days until the first egg hatches (Simmons 2000; Simmons et al. 2005). A brood holds from 1 to 4 eggs, usually 3 (3.5 on average, $n = 58$ nests; with only 2 records of 5 eggs in 16 years; Simmons et al. 2005). The first two eggs generally hatch synchronously, followed by asynchronous hatching of the third and later eggs, sometimes with the third egg hatching up to 12 days after the first one (MSGH *personal observations*); this results in large size differences between chicks. Black Harrier females provide the parental care of nestlings at the nests and perform all brooding until nestlings are about 20-25 days old. At that stage, females also start capturing and providing prey, the latter responsibility being typically the role of the males in the early nestling period (Simmons 2000; Redpath et al. 2002a). Individuals are solitary foragers and typically forage 1-3 m above vegetation, with buoyant long-distance (i.e. between 9-18 km covered on

foraging trips according to data from GPS-transmitters tagged birds from 2009-2015; (RES, *unpublished data*) flap-sailing flight interspersed with occasional hovering (Simmons et al. 2005).

Like other raptors, Black Harriers are sexually dimorphic, females being around 7 % larger than males in wing length, and approximately 28 % heavier than males (*unpublished data*). On the other hand, and in contrast to all Northern Hemisphere harrier species, the Black Harrier is not sexually dichromatic. This is a trait common to most of the Southern Hemisphere harriers (Simmons 2000). Both adults have an identical pied plumage, with the upperparts and underparts almost entirely black. The primaries and secondaries are white on the ventral surface, the edging on all remiges is black, and the tail has a striking black and white banding. Juveniles of both sexes are dark brown, with a pale belly. Adults and nestlings have a black bill and both display a carotenoid-pigmented cere and tarsus, which ranges from a white-yellow pale colour at birth, towards a very bright yellow-orange colouration as nestlings' mature and at the adult stage. Nestlings have dark eyes that lighten at the juvenile stage before becoming bright yellow at the adult stage (Simmons et al. 2005).

Black Harrier – previous research

Here, I first give a general outline of the characteristics of the areas where Black Harriers' breed, together with information on existing population monitoring programs. I further summarise previous research on the species, and highlight the knowledge gaps that motivated my PhD research.

Black Harriers' breeding areas

Black Harriers breed in indigenous vegetation, essentially along the South African coast within the Fynbos biome, and inland within the Succulent and Nama-Karoo biome (Curtis et al. 2004; Curtis 2005).

The Nama-Karoo biome is the largest biome in South Africa which occupies most of the interior of the western half of the country and covers about 20.5% of the surface of the country, i.e. approximately 260 000 km² (Figure 3) (Palmer & Hoffman, 1997). The Nama-Karoo biome is considered a semi-desert, with altitudes between 500-2000m, although

most of the biome falls between 1000 and 1400m. Rainfall is highly seasonal, peaking between December and March, and varies between 60 mm in the west and 400 mm in the north-east. Summers are usually very hot (Max-mean: $>30^{\circ}\text{C}$), winters are very cold with temperatures below freezing, and daily variation is as large as 25°C between day and night (Palmer & Hoffman, 1997). Overall, the Nama-Karoo biome is considered as a xeric shrubland with many deciduous plants. To be more precise, its vegetation is characterized as a dwarf open shrubland or open dwarf-shrub steppe, dominated by Asteraceae, Poaceae, Aizoaceae, Mesembryanthemaceae, Liliaceae and Scrophulariaceae (Palmer & Hoffman, 1997). Trees like the Sweet Thorn *Vachellia karroo* are usually only found along rivers or on rocky hillsides. Additionally, the Nama Karoo biome is famous for sheep and goat farming, and in the main river valleys, people also farm olives, citrus and deciduous fruit trees.

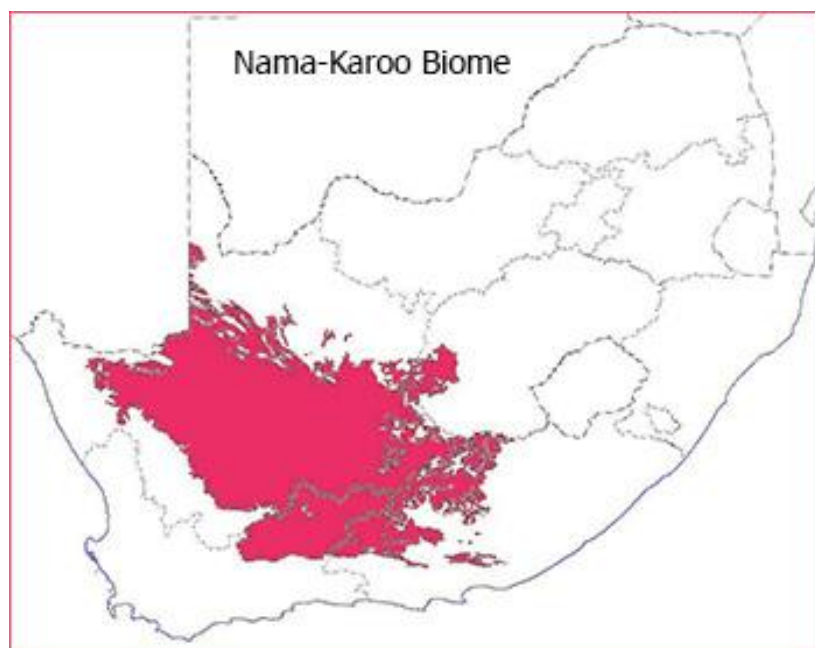


Figure 3. Distribution of the Nama-Karoo Biome within South Africa. Map extracted from the South African National Biodiversity Institute (SANBI; <http://pza.sanbi.org/vegetation/nama-karoo-biome>).

The Succulent Karoo biome covers the arid western parts of South Africa, which represents approximately 7.5% of the surface of the country, i.e. 83 000 km² (Milton et al. 1997) (Figure 4). Comprising coastal plains and inter-mountain valleys, it is located along

South Africa's Northern Cape Province and extends inland into the uplands of South Africa's Western Cape Province. Altitude is mostly below 800m but in the east it may reach 1500m. The region is extremely dry in summer (November-March) and is characterized by low annual rainfall, i.e. 20-290 mm per year. Temperatures often rise above 40°C during the day (Milton et al. 1997). The Succulent Karoo has the highest richness recorded for any semi-arid area, and is considered to be the world's richest flora of succulent plants, with more than 50% of the plant species being endemic to this biome (Cowling et al. 1989, 1997). Chamaephytes and geophytes are predominantly found, although there is a scarcity of tall shrubs, trees and grasses. Overall, plants within this biome are adapted to survive extremely dry summers, e.g. annual daisies and geophytes remain dormant in summer and grow and flower after the winter rains (Cowling et al. 1997). Many parts of the Succulent Karoo biome are famous for their spring flowers, where the flower tourism is becoming an important source of income for these regions.

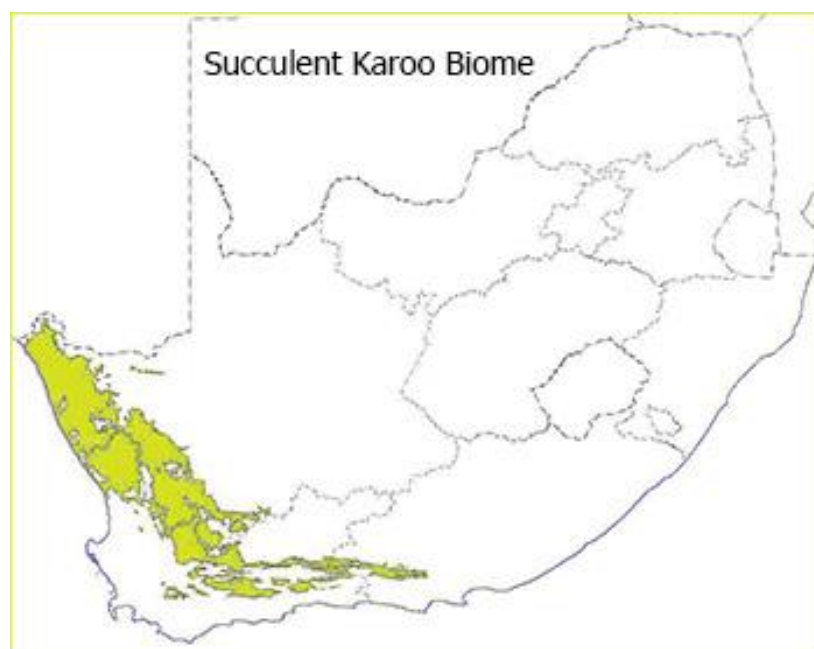


Figure 4. Distribution of the Succulent Karoo Biome within South Africa. Map extracted from the South African National Biodiversity Institute (SANBI; <http://pza.sanbi.org/vegetation/succulent-karoo-biome>).

The Fynbos biome extends across south-western South Africa in a 100-200 km wide coastal belt in the Western Cape Province, an area covering approximately 6.7% of the surface of the country, i.e. 85 000 km² (Cowling et al. 1997) (Figure 5). Its predominant vegetation type is Fynbos, an evergreen, fire-prone shrubland, confined largely to sandy, infertile soils and characterized structurally by the universal presence of restioids (wiry, evergreen graminoids) and by a high cover of ericoid shrubs (specially Ericacea) (Cowling et al. 1997). The Fynbos Biome forms the main part of the Cape Floristic Region, which includes a mosaic of diverse habitat and vegetation types, many of which are nationally and internationally protected and considered of high biological and ecological values (see e.g. Manning 2007; Mucina & Rutherford 2006). The Cape Floristic Region is indeed considered as a biodiversity hotspot of global significance due to its species richness and high endemism of plants and animals (Siegfried 1992; Picker & Samways 1996; Cowling & Pressey 2003; Cowling et al. 2003). Numbering almost 9000 species of flowering plants, two thirds of them are endemic to this region. As a result the Cape Floristic Region has been recognized as one of the six world's richest flora kingdoms regions (Cowling & Richardson 1995; Cowling et al. 1997; Myers et al. 2000). The region is characterised by a Mediterranean climate with cold and wet winters (May-October), and warm and dry summers (November-March) (South African Weather Services 2015). Rain falls mainly in winter (i.e. May/June to October/November), and rainfall varies from about 210 mm in the inland valleys, to about 400 mm in the broad coastal forelands and about 800-3000 mm in the mountains. Unfortunately, its mild and temperate climate (mean annual temperature of 16-20°C) also contributes to the vulnerability of this ecosystem: the region is relatively densely populated and has been heavily impacted by anthropogenic modifications of land use during the second half of the last century, resulting in the destruction and fragmentation of natural habitats (Cowling et al. 1997). Black Harrier's overall breeding distribution is located within the Cape Floristic Region, and as a consequence, the species' breeding habitats were reduced by 50 % during the last century, and many nesting areas are now surrounded by agricultural lands (i.e. cereal agriculture and viticulture), sheep farming and urbanization (Allan 1993; Low & Rebelo 1996; Kemper et al. 2000). The spread of alien vegetation and introduced species, the increase of plantations, and the overharvesting and exploitation of natural resources in this region has raised (and continues to highlight) concerns over the

conservation status of the Cape Floristic Region since the early 1900s, when the high biodiversity of threatened and endemic flora and fauna in the region was recognised (Wicht 1943; Cowling & Richardson 1995).

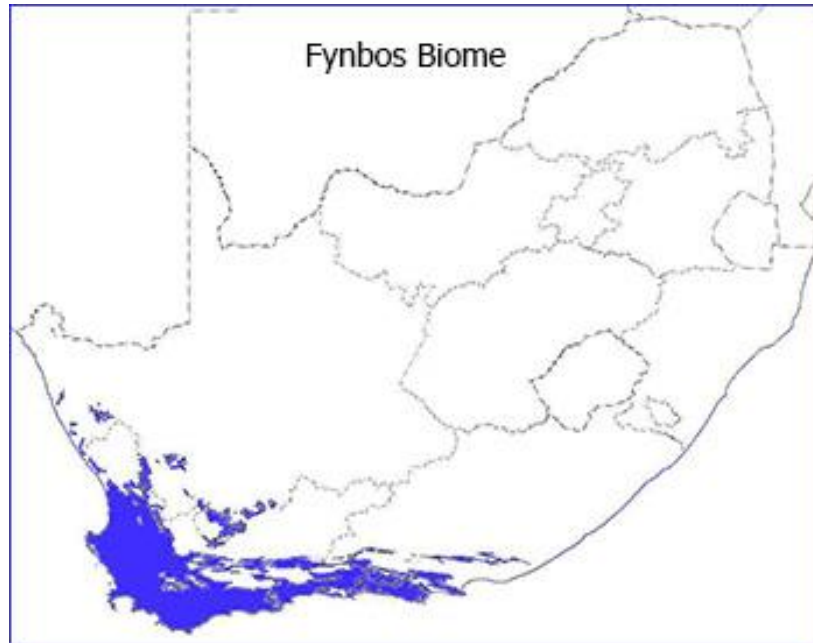


Figure 5. Distribution of the Succulent Karoo Biome within South Africa. Map extracted from the South African National Biodiversity Institute (SANBI; <http://pza.sanbi.org/vegetation/fynbos-biome>).

Existing monitoring programs

The first review on the status and biology of the Black Harriers was published by Van der Merwe in 1981. His work assembled and collated all unpublished and published observations on the species sporadically collected since the early 1900s. His was the first synthesis of this over-looked harrier and took our knowledge of the species from a series of anecdotes to a much clearer picture of the bird's ecology and habitat preferences. However, the regular monitoring of the Black Harrier breeding population only really started in 2000, initiated by Drs. R.E. Simmons and A.R. Jenkins. Among others, data on breeding biology and diet were collected over the years 2000-2011 within the species' breeding range. Breeding sites were located within the Western, Northern and Eastern Cape Provinces of South Africa, in and around National Parks (i.e. South African National Parks, such as the Table

Mountain, West Coast, Bontebok, de Hoop, or Karoo National Parks), provincial protected reserves (i.e. Cape Nature), or on private lands. Four studies of Black Harriers were undertaken during this time, the first a two-year study by Odette Curtis assessed the influence of habitat fragmentation on the ecology and reproductive success of harriers in the Overberg and in coastal areas (Curtis 2005). The second year-long study by Julia Jenkins assessed the role Black Harriers might play as indicators of biodiversity, measured in terms of avian, plant and mammal diversity, in the Cape Floral region (Jenkins et al. 2013). Short-term honours projects by Marion Atyeo and Kirsten Retief added to our knowledge of the Black Harriers habitat choice in relation to small mammal populations and their foraging behaviour in relation to small mammal activity patterns (Atyeo 2006; Retief 2011).

More intense field work was conducted for my PhD during the 2012-2015 breeding seasons, covering both coastal and inland nests. During these years, the “coastal” study region included all the breeding sites located from the Koeberg Nature Reserve (33.675° S, 18.433° E, about 35km north of Cape Town), towards the West Coast National Park (33.169° S, 18.092° E). These breeding sites were located along the west coast of the Western Cape Province, within the Fynbos biome, spanning a distance of about 70 km. I contrasted this with a sample of nests from an “inland” region over these four years of study, that included breeding sites located in the surroundings of Nieuwoudtville (31.316° S, 19.083° E), Vanrhynsdorp (31.420° S, 18.708° E), Calvinia (31.420° S, 19.765° E) and Papkuisfontein Private Nature Reserve (31.543° S, 19.150° E). The majority of these breeding sites were located in the Northern Cape Province, within the Nama and Succulent Karoo biomes. Additional information about the regions is described in each of the chapters, where necessary. The geographical location of the coastal and inland regions monitored during 2012-2015 are shown in Figure 6.

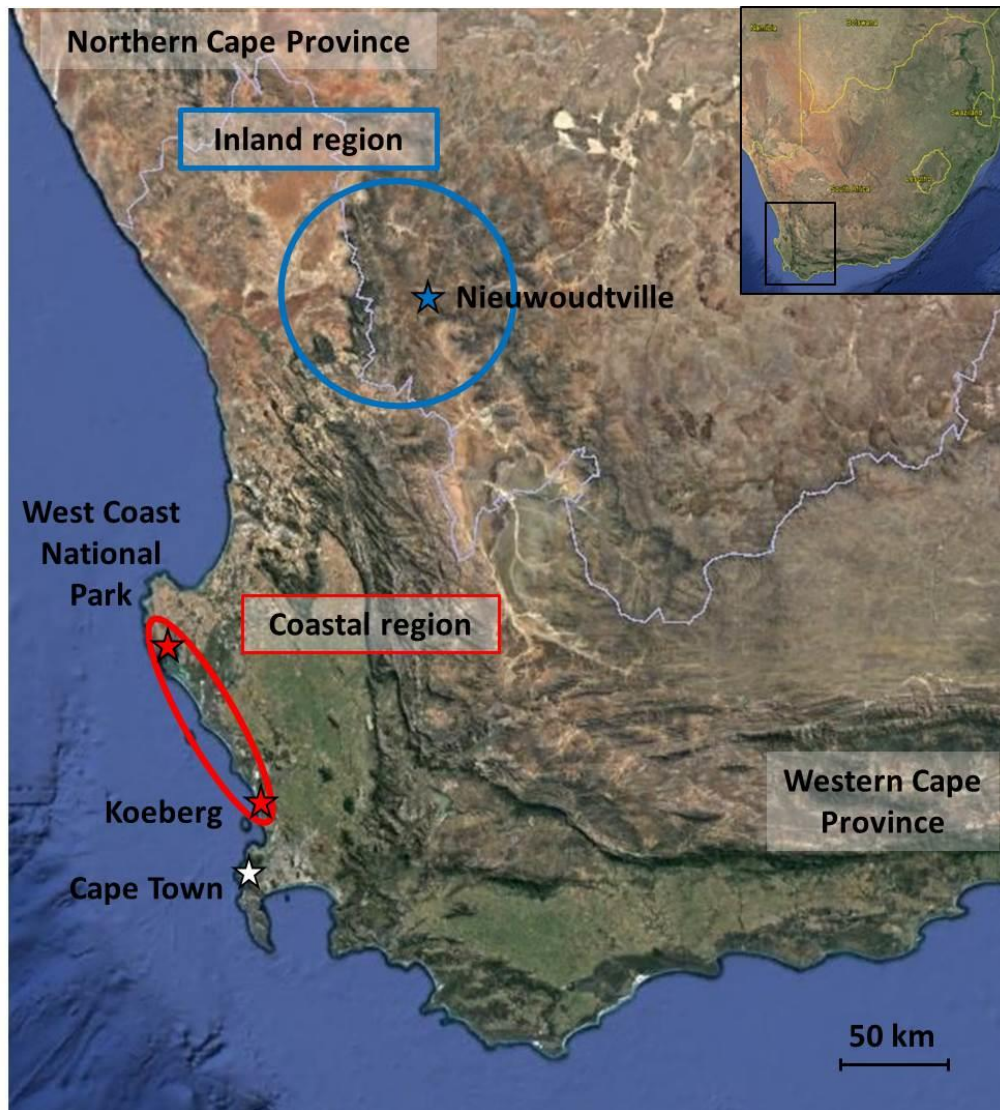


Figure 6. Location of the coastal (red ellipse) and inland (blue circle) regions where intense fieldwork was conducted in 2012-2015 for the purpose of this PhD. Important locations are represented with colour stars: white for the city of Cape Town; red for the two main study sites at the coastal, Koeberg Natural reserve and the West Coast National Park; blue for the town of Nieuwoudtville.

Existing research

The loss and fragmentation of Black Harriers' natural breeding habitats have been suggested as one of the main factors explaining the current scarcity of the species. Black Harriers essentially hunt in natural vegetation, and whereas they also often hunt in agricultural habitats, mostly in the inland regions, only two records have mentioned breeding Black Harriers in cultivated areas (Steyn 1982; Chadwick 1997). This suggests that the species is

not capable of adapting to breed in non-natural environments (Curtis et al. 2004; Jenkins et al. 2013), contrary to Palearctic Harriers (Arroyo et al. 2002; Millon et al. 2002), or other south African raptors such as Verreaux's Eagles *Aquila verreauxii* (Murgatroyd et al. 2016b) or the Black Sparrowhawk *Accipiter melanoleucus* (Malan & Robinson 2001). In her master thesis, Curtis (2005) addressed these issues by investigating the impacts of habitat fragmentation on breeding Black Harriers. She suggested that breeders were sensitive to patch size, with no breeding Black Harrier found in patches with less than 450 ha of natural vegetation within a 3km radius around the nest. She suggested that this might be the minimum area of un-fragmented natural habitat required in the proximity of the nest to allow breeding. This also supports the hypothesis that the species may have been negatively impacted by habitat fragmentation. Furthermore, Curtis (2005) also demonstrated that in areas where habitat fragmentation was more pronounced, active nests were more dispersed, and breeders more territorial. By contrast, in areas supporting larger patches of natural habitats with less habitat fragmentation, such as the west coast, Black Harriers breed closer to each other in a less dispersed semi-colonial fashion. Although these implied that coastal regions were more appropriate for breeding Black Harriers, it was also suggested that breeding in clumps could have negative impacts due to more intra-specific competition over food which ultimately may reduce an individuals' breeding success. Finally, in order to determine if Black Harriers were able to harvest all their food resources within the study area (i.e. Bontebok National Park), Curtis (2005) fitted two adults with radio-tracking devices in 2004. Her results suggested that, indeed, both Black Harriers could secure all their food within the boundaries of the park (3,900 hectares), and concluded that intact vegetation patches can provide a nest site and all the foraging needs of a harrier pair.

On the other hand, detailed analysis of breeding phenology and reproductive success, as well as on the diet composition of Black Harriers during the breeding period were particularly limited, although they are key parameters to understanding a species population ecology and basic conservation requirements. Precise information about variation in the timing of breeding between years or regions was also entirely lacking. Similarly, the species has been tentatively described as a small mammal specialist (Van der Merwe 1981; Steyn 1982), although quantitative information on the bird's diet was scarce. Curtis et al. (2004) suggested that Black Harrier's diet may vary with geographical location:

individuals breeding along the coast appeared to feed primarily on small mammals, while those breeding inland, in mountainous or lowland areas, had a more diverse diet, including more birds (Curtis et al. 2004). Their study was based on a relatively small sample size over three years from 2000-2002, so detailed information about the diet of this Endangered species, and spatial-temporal (i.e. annual or regional) variations was also overall lacking. While Curtis et al. (2004) explored variations in breeding success using data from those 3 years (2000-2002), their analyses did not account for variations in the timing of breeding or the influence of factors potentially influencing food abundance, such as weather. In this context, it appeared to be relevant to conduct further research on breeding phenology and success, diet composition and its relationship with geographical variation, and weather conditions to help explain the scarcity of the species, if optimal conditions for breeding are restricted in space.

Recent satellite- and GPS-tagging of adult Black Harriers has revealed a migration towards the east of South Africa in summer following breeding in spring (see blog of RES: <http://blackharrierspace.blogspot.co.za/2015/01/the-season-for-migration-east.html>, *unpublished data*; Taylor 2015). As the breeding period ends, adult Black Harriers migrate towards Lesotho, the Eastern Cape and the Kwazulu Natal Provinces, coincident with the summer rains there, travelling as far as 1200 km. There, they are found within the Sweet Grassveld biome, the mixed sub-tropical/Afromontane forest, and the Arid savanna and bushveld biome, where they spend about 6 months before heading back to the western regions of the country to breed again, coincident with the winter rains. Thanks to tagged adults, new breeding areas have been discovered which increased the number and known distribution of Black Harrier breeding site. These data also show that birds may move considerable distances between consecutive breeding attempts (see blog).

Finally, a recent study of the mitochondrial DNA in moulted adult Black Harrier feathers showed no genetic diversity within the population, suggesting a bottleneck in the past. This concurs with the small population size estimated for the species (Fuchs et al. 2014; but see contrary example found by Johnson et al. (2009) with the Madagascar Fish-Eagle *Haliaeetus vociferoides*). This may contribute to the species being highly vulnerable to environmental change, climate change, pathogens, or contaminant exposure. To date, there

is a total lack of knowledge concerning the “health status” of Black Harrier, and if and how organic pollutants may be affecting Black Harrier individuals’ health or breeding. Urgent research on this topic is, therefore, needed to identify the potential sources of contaminant exposure to Black Harriers and investigate the potential sub-lethal effects on individuals as this may indirectly be a contributing factor explaining the scarcity of the species.

THESIS OVERVIEW

The overall approach of my PhD dissertation is multifaceted, as it includes general concepts of population ecology, as well as other disciplines such as eco-toxicology and eco-physiology. My research attempts to determine how biotic and abiotic factors may potentially affect wild Black Harriers, and ultimately whether these factors may potentially explain the scarcity of the Black Harrier population in southern Africa. I collected most data during the 2012-2015 breeding seasons, however, for Chapters 1, 2 and 3, I also analysed historical data collected by RES during the 2000-2011 breeding seasons. A schematic representation of the PhD outline is given in Figure 7.

Investigating how breeding parameters vary spatially and temporally is central to population ecology and is particularly important for species of conservation concern (Newton 1979, 1998; Krebs 1985). Understanding variation in the timing of breeding and its potential fitness consequences is an essential intermediate step, as it may reveal limiting factors for the species through assessing conditions associated to the optimal time for breeding (Perrins 1970; Verhulst & Nilsson 2008). In this context, in Chapter 1, I explore at the population level, how key breeding parameters (clutch size, breeding success and productivity) vary with the timing of breeding, weather conditions (temperatures and rainfall) and between regions (coastal vs. inland), using data from ca. 400 breeding events collected from the 2000-2014 breeding seasons. Chapter 1 has been published as a paper in *Nature Conservation* (Garcia-Heras et al. 2016a).

In Chapter 2, I examine diet composition of the species during the breeding period. Understanding the feeding habits of animals and assessing the breadth and availability of food resources use is at the core of ecological research (Martínez del Río et al. 2009). Food

supply is often the main factor affecting population densities of many bird species, including raptors (Newton 1979). The study of diet provides basic background information to understand food requirements, and also dietary flexibility (the position along the specialist vs. generalist spectrum), which is a crucial step in understanding any species' ecology (Arroyo 1997). Using ca. 1,000 pellets (> 1,700 identified prey) collected at nest sites, I describe the diet composition to the species level (when possible) in all the monitored areas over 10 breeding seasons from 2006-2015. Chapter 2 has been published as a paper in *Ostrich: Journal of African Ornithology* (García-Heras et al. 2017a).

For species of conservation concern, it is paramount to identify under which conditions food is limiting and to characterize the factors shaping a species' dietary niche or food availability. The latter is mainly determined by both the abundance and accessibility of food resources (Preston 1990; Newton 1998). In Chapter 3, I examine the spatial-temporal variation in diet, and its relation with factors potentially affecting prey abundance (e.g. winter rainfall which potentially affects primary production) or accessibility (e.g. daily temperatures which potentially affects prey behaviour). Using the pellet data described above in Chapter 2, I explore whether diet composition changes throughout the breeding season in both the coastal and inland regions. Additionally, using camera recordings during the 2014 breeding season (n= 1557 videos and 90,692 minutes of recording time), I further report regional differences in the daily patterns of prey delivered to nestlings, that may reflect differences in prey accessibility between the two regions. Finally, I also show that regional differences in seasonal trends in diet composition are explained by variations in maximum temperature, indicating that high summer temperatures may limit accessibility of the main prey. While awaiting for the examination of this thesis, this chapter has been published in the *Ibis* journal (García-Heras et al. 2017b).

In Chapters 4 and 5, I use an eco-toxicological and eco-physiological approach to assess environmental contamination and its potential effects on the health and condition of wild adult and nestling Black Harriers. In Chapter 4, I first report on the occurrence and patterns of Organochlorine Compound (OCs) contamination, such as polychlorinated biphenyl (PCBs) and dichlorodiphenyltrichloroethane (DDTs: p,p'-DDT and its metabolite p,p'-DDE). I further report on correlates of OC concentrations in order to precise potential

pollutant sources, such as variations with respect to habitat types within the territory, electric transformer density, and diet composition. Finally, I explore whether these OCs may be affecting the physical or physiological condition of harriers. This was achieved through analyses of a body condition index (mass corrected for size or age), and white blood cell count and heterophils to lymphocytes ratios, respectively. For the latter, I used blood samples collected during the 2012-2014 breeding seasons, from 90 nestlings and 24 adults. While awaiting for the examination of this thesis, this chapter was submitted to the *Environmental Pollution* journal, and is currently under second revision.

The goal of Chapter 5 was to examine whether these OC (Σ PCB and Σ DDT) concentrations could affect the circulating carotenoid levels and integument colouration of Black Harrier nestlings. Carotenoids are indeed important components in avian physiology. They serve important health-related physiological functions, acting as antioxidants to limit oxidative stress and oxidative damage, and can boost the immune system through immunostimulation and immune-regulation functions. Also, carotenoid-based traits or ornaments play key roles in bird communication and in social interactions. A disruption of these traits by OCs may have broader implications for social communication. I investigated the colouration of the cere and tarsi, as the expression of carotenoids in fleshy integuments is a dynamic trait that may rapidly fluctuate due to external changes (Velando et al. 2006). Such a change in colouration and circulating carotenoid levels may reflect recent changes in individual condition (e.g. Faivre et al. 2003; López et al. 2011). Using data collected during the 2012-2014 breeding seasons, I first examine the relation between the yellow-orange colouration of the cere and tarsi, and the blood circulating carotenoid levels in nestlings according to age, sex, brood rank, physical condition and dietary composition. Second, I tested whether circulating carotenoid and coloration varied with blood-DDT and PCB levels. While awaiting for the examination of this thesis, this chapter has been published in the *Science of Total Environment* journal (García-Heras et al. 2017c).

Finally, I discuss in my synthesis the research findings from Chapters 1-5, highlighting the importance of multifaceted studies when attempting to understand the factors limiting a species' population size, while emphasising the importance of taking into account both the population and the individual levels. In doing so, this study provides a better

comprehension of which environmental and/or individual parameters may affect the breeding and health condition of a population, which in turn may infer potential reasons responsible for its scarcity. This will help in implementing more efficient conservation strategies for this Endangered avian predator species in the long term.

Brief note on chapter structure

Each chapter is written as a stand-alone paper to facilitate the publication of the work and therefore each chapter comprises an abstract, introduction, materials and methods, results and discussion section. As such, there is some inevitable repetition of concepts and information throughout the introductory, method and discussion sections of these chapters. Inconsistencies may also occur between chapters: for example the use of the term “coastal and inland regions” in chapter 1-3 refers to the overall characterisation of nests monitored from 2000-2015 within Black Harrier entire breeding range, while in Chapters 4 and 5 it specifically refers to the two main study areas, where I have intensively collected data from 2012-2014. Each term is clarified in the methods section of respective chapters. Within these data chapters I use the term “we”, since there were several authors on each paper, although in all cases I am the principal lead author. All the references have however been placed at the end of the thesis. Finally, where chapters have been accepted or submitted for publication, the text has been edited and formatted to fit with the rest of the thesis.

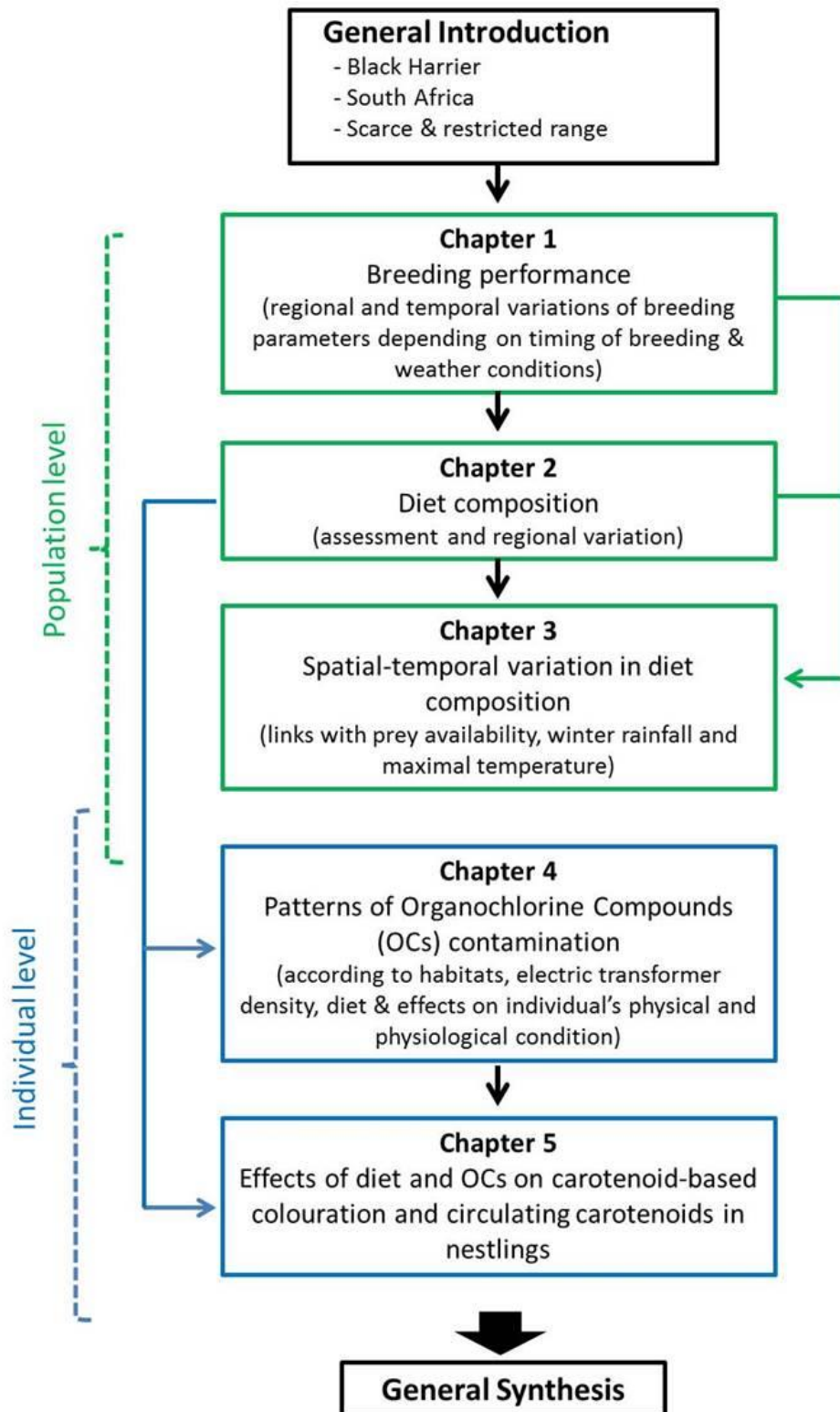


Figure 7. Schematic representation of the PhD thesis outline. For each chapter, the general context was given, and the link between chapters is illustrated with arrows. Green colour chapters were conducted at the population level, while blue colour chapters were conducted at the individual level.

Chapter 1

Does timing of breeding matter less where the grass is greener? Seasonal declines in breeding performance differ between regions in an Endangered endemic raptor



A modified form of this chapter has been published in Nature Conservation as: *García-Heras M.-S, Arroyo B., Mougeot F., Amar A. & Simmons R.E. 2016. Does timing of breeding matter less where the grass is greener? Seasonal declines in breeding performance differ between regions in an endangered endemic raptor. Nature Conservation, 15: 23-45.*

ABSTRACT

Timing of breeding can strongly influence individual breeding performance and fitness. Seasonal declines in breeding parameters have been often documented in birds, particularly in the Northern Hemisphere. Fewer studies have investigated whether seasonal declines in productivity vary in space, which would have implications for a species' population dynamics across its distributional range. We report here on variation in the timing of breeding in the Black Harrier *Circus maurus*, an Endangered and endemic raptor to southern Africa. We investigated how key breeding parameters (clutch size, nesting success and productivity) varied with the timing of breeding, weather conditions (rainfall and temperatures) and between contrasting regions (coastal vs. inland). The onset of breeding extended over an 8-month period, with a peak of laying between mid-August and end of September. A marked seasonal decline was apparent in all breeding parameters. Importantly, for clutch size and productivity these seasonal declines differed regionally, being more pronounced in inland than in coastal regions, where the breeding season was overall shorter. Timing of breeding, clutch size and productivity were also partly explained by weather conditions. In coastal regions, where environmental conditions appear to be less variable, the timing of breeding matters less for breeding output than in inland regions, and breeding attempts thus occurred over a longer period there. The former areas may act as population sources and be key in protecting the long-term population viability of this threatened raptor. This study provides unique evidence for a regionally variable seasonal decline in breeding performance with implications for population biology and conservation.

INTRODUCTION

Understanding spatial-temporal variations in breeding parameters is an essential component of population ecology, and is particularly important for species that are of conservation concern, as this may help identify reasons for population decline and scarcity (Newton 1979, 1998; Krebs 1985). In this context, understanding variation in the timing of breeding and its potential fitness consequences is an essential intermediate step, and may reveal limiting factors for the species (Perrins 1970; Verhulst & Nilsson 2008). Quality of the breeding area, predation risk, inter- and intra-specific competition, individual quality and time of migration (Newton 1998) have all been found to affect timing of breeding in bird species. Overall, weather conditions (Charmantier et al. 2008; Visser et al. 2009) and food abundance (Newton 1998; Verboven et al. 2001) are generally considered the two main drivers influencing variation in the timing of breeding in bird species. In tropical birds, breeding onset may occur throughout the year as a result of a less seasonal climate and more constant food availability and abundance (Simmons 2000; de Marchi et al. 2015). At the other extreme, breeding onset in Arctic species depends on snow cover in spring and is restricted to a very narrow temporal window (Dickey et al. 2008).

The timing of breeding is a key determinant of breeding success and productivity (e.g. Verhulst & Nilsson 2008; Dunn & Møller 2014; Martin et al. 2014). In many woodland passerines, laying usually occurs so that the nestling period matches the seasonal peak in caterpillar abundance, which in turn is determined by weather conditions, such as temperatures in spring (Lof et al. 2012). Breeding too early or too late in relation to optimal conditions may lead to lower breeding performance (Robb et al. 2008). Seasonal declines in breeding outputs have been observed in many species, with birds breeding earlier in the season having higher reproductive outputs than those breeding later on (Verboven & Visser 1998; Mougeot & Bretagnolle 2006; Verhulst & Nilsson 2008). This pattern may arise when individuals breeding earlier in the season are of better quality, and/or when environmental conditions degrade as the season progresses (e.g. worsening weather conditions, reduced food abundance and quality, poorer quality breeding habitat vegetation; Verhulst et al. 1995; Verhulst & Nilsson 2008). The latter scenario implies that optimal conditions for breeding are temporally limited within a breeding season. In a context of climate change

and rapidly changing environmental conditions, a pre-existing synchrony between the timing of breeding and the availability of key breeding resources (seasonal food peak) may be disrupted leading to biodiversity loss (Visser et al. 2004), but more investigation is needed on this topic (e.g. Visser & Both 2005; Reed et al. 2013; Grimm et al. 2015).

Recent research has also indicated that seasonal declines in breeding performance may vary in strength depending on habitat type or location. For example, Zárbynická et al. (2015) found that Tengmalm's Owl *Aegolius funereus* showed different seasonal declines in productivity in temperate and boreal areas, principally due to differences in nestling mortality rates across the season. In the Great Tit *Parus major*, clutch size declines through the breeding season have been reported in rural, but not in urban areas (Wawrzyniak et al. 2015). This may imply that conditions for breeding in the latter habitat are more stable or last longer in the year, which may have implications for the ecology of these populations. However, with these few exceptions, the variability in declines of seasonal reproductive performance remains poorly studied or explored.

Research on the relationship between timing of breeding (i.e. lay date) and breeding output (e.g. clutch size, success or productivity) in birds, to date, has been mainly conducted in temperate and boreal regions (Barnard et al. 1987; Amar et al. 2012; Dunn & Møller 2014). Relatively few studies have explored the association between timing of breeding and breeding outputs in the Southern Hemisphere, particularly in Africa (Simmons 2000; Lepage & Lloyd 2004; Martin et al. 2014; Murgatroyd et al. 2016b). Identifying these associations may contribute to our understanding of why some populations are more or less successful under certain circumstances and conditions than others. This may be particularly important when dealing with Endangered species, as it may allow prioritizing conservation efforts of target species in space or time (Green et al. 2006; Amar et al. 2008; Gangoso et al. 2009).

The Black Harrier *Circus maurus* is a ground-nesting medium-sized bird of prey, endemic to southern Africa. The species is very scarce with an estimated total world population of less than 1,000 mature breeding birds, a distribution range of approximately 500 000 km² and a highly restricted breeding range of approximately 170 000 km² (Van der Merwe 1981; Siegfried 1992; Simmons 2000; Taylor 2015). The species is listed as Endangered in South Africa, Namibia and Lesotho (Taylor 2015; Simmons et al. 2015), these three countries

encompass the totality of its breeding range. Black Harriers is known to essentially breed in south-western South Africa, along the coast and more inland (Curtis et al. 2004; Curtis 2005), but the species remains understudied (Van der Merwe 1981; Simmons et al. 1998; Curtis et al. 2004; Curtis 2005; Simmons et al. 2005; Jenkins et al. 2013) and key information on factors limiting breeding parameters is scarce. Curtis et al. (2004) explored variation in breeding parameters between nests in coastal and inland regions, finding that clutch sizes and productivity were greater in coastal regions. However, this study was based on data from only 3 years (2000-2002) and their analyses did not account for variations due to the timing of breeding or the influence of weather. Black Harriers are known to lay clutches over an extended period (from mid-May to mid-December, Simmons et al. 2005), but information on variation in the timing of breeding between years or regions is currently lacking. The breeding range of the Black Harrier mainly coincides with the Mediterranean climate zone of South Africa, characterized by cold and wet winters (May-September), and warm and dry summers (October-April). The seasonal fluctuations characterizing this climatic zone may influence the timing of breeding for Black Harriers, which may also differ between the main nesting regions.

In this study, we use a large data set of nearly 400 breeding events of this scarce species collected over 15 years (2000-2014) in South Africa to investigate spatial-temporal variations in breeding performance. We first report on regional variation in the timing of breeding, and its association with weather conditions (i.e. rainfall and temperature). We then investigate whether key breeding parameters, i.e. clutch size, nesting success and productivity, vary depending on the timing of breeding, geographical location (coastal vs. inland regions) and weather conditions. Lastly we evaluate whether seasonal declines in breeding performance differ in strength between regions, and the potential implications this might have for the conservation of this species.

MATERIALS AND METHODS

Study area

Breeding data were collected opportunistically over a large area (ca. 170,000 km²) of temperate south-western South Africa (-29° S, 17° E; -34° S, 27° E) from 2000 to 2011. More focused studies took place along the west coast of the Western Cape Province and inland in the Northern Cape Province around Nieuwoudtville (-31.316° S, 19.083° E) first from 2000 to 2002, and then from 2012 to 2014. Nests were located in and around national parks (i.e. South African National Parks - SANParks), provincial protected reserves (i.e. Cape Nature), or on private lands. They were spread across a mosaic of different biomes with diverse habitats and vegetation types, many of which are nationally and internationally protected and considered of high biological and ecological values (see e.g. Manning 2007; Mucina & Rutherford 2006). Climate across the study area varies between provinces: the west of the Eastern Cape, and Western Cape have a more temperate climate and a winter rainfall regime (April to September), while the coastal Northern Cape also experiences a winter rainfall regime but with more fluctuating temperatures (South African Weather Services 2015).

Breeding sites were located by observing areas where Black Harriers were previously known to breed and/or where perched adults were detected. As in other raptor species, the females take care of the chicks at the nests and perform all brooding, while the male captures and provides the food in the early nestling period (Simmons 2000; Redpath et al. 2002a). Thus, nests were located by following prey-carrying males and observing where females landed after a food pass (Simmons 2000).

Breeding parameters

After discovery, nests were visited regularly (usually 2-3 times per breeding event) where possible to assess nesting success and productivity. However, because of the extensive nature of the study area, not all breeding areas and nest sites were monitored consistently each year, and for some remote areas, nest sites were only visited once, or were last visited prior to fledging. During each nest visit, we noted the nest contents (i.e. number of eggs or nestlings) and, if the nests contained nestlings, a visual estimate of age was taken. In a

subsample of nests, wing, tail and tarsus length (mm), and mass (g) of chicks were measured. Nest visits were kept as brief as possible (< 20 min) and an effort was made to leave the vegetation around the nest undisturbed. The location of nests was recorded using a Garmin global positioning system (GPS). A total of 490 nests were located between 2000 and 2014, although not all variables examined in this study were available for each breeding attempt, so sample size varies among analyses.

Lay dates were estimated either with the presence of bluish (newly-laid) eggs (R.E.S. personal observation), or by subtracting 31 days (Simmons et al. 2005) from hatch date, which was in turn estimated either (i) directly when a clutch was found with an egg hatching or (ii) from a newly hatched chicks (aged 1-3 days old) or (iii) indirectly from nestling age. Nestling age was estimated either visually (see above) or through body measurements using data from a subsample of nests that were visited more regularly. From these nests we constructed growth curves of wing-length and age for this species (see Appendix A). Given the variation in precision of lay dates among nests, for all analyses we attributed the laying date for each nest to a 15-day period (where 1= 1-15 May, 2= 16-31 May, etc., up to 15= 1–15 December). For the sake of simplicity, we henceforth refer to these lay date periods as “lay date” even though they are not exact dates. Lay date could not be determined for nests located during the incubation period and visited only once or that failed before the second visit (n= 70), or for nests discovered after fledging or for breeding records without a precise visit date (n= 18); therefore, data from these nests were excluded from the breeding phenology analyses. Overall, lay date was estimated for 402 breeding events.

Clutch size was defined as the maximum number of eggs laid. When possible, nests were visited twice during the incubation period with the second visit timed to coincide with the estimated date of hatch. This ensured that we recorded the exact number of eggs laid per breeding event. Nests that were visited before the clutch was finished and that subsequently failed, only during the nestling period were excluded from clutch size analyses. Exact clutch size was known for 191 breeding attempts.

Breeding output was measured in two ways, nesting success (known for n= 263 breeding attempts) and productivity (n= 261). Nesting success was classified as 1 for those nests where at least one young was raised to 35 days old, or 0 otherwise. Productivity was

defined as the number of young reaching 35 days of age (range 0-4) for pairs that laid a clutch. Black Harriers fledge at approximately 40 days old (Simmons et al. 2005) but in many cases our last visit occurred before that age; however, in harriers, as in many other species there is usually little mortality during this late nestling stage (Redpath et al. 2002a). Thus, we assume that all nestlings alive at 35 days old fledged.

Topographic parameters

Nest coordinates were incorporated in a geographical information system (QGIS Valmiera 2.2.0), projected on WGS84-UTM-34S as the coordinate reference system. Using this GIS, we calculated and identified the following variables for each nest: i) Altitude, from the Shuttle Radar Topography Mission (SRTM) 90 m Digital Elevation Database v4.1 (Srtm90m). ii) Region (coastal and inland) was defined using a combination of nest altitude (from SRTM) and distance to the coast. Coastal nests were defined as those located within 15 km from the coast and with a maximal altitude of 100 mASL (n= 328). Nests located further than 15 km from the coast and with an altitude higher than 100 mASL were considered as inland (n= 146). However, this classification excluded nine nests that were located higher than 100 mASL (average of 118 m), but within 15 km from the coast and for the purpose of our analysis these were classified as coastal. Another eight nests were located at an altitude lower than 100 m, but 45 km from the coast, and these were classified as inland. In both cases, we believe our classification to more accurately describe conditions for those 17 nests. This regional classification was initiated by Curtis et al. (2004) to explore regional differences in lay dates and productivity of Black Harriers. That study also further differentiated between nests in mountain and interior-lowland areas. However, overall sample size of interior-lowland areas was too small to allow meaningful comparisons, and so these two categories were grouped as a single region, i.e. inland, for our study.

Weather data

Weather data were obtained for the period 2000-2014 from 17 weather stations distributed throughout the study area (source: South African Weather Services 2015) (Figure 8). For some stations, weather data were lacking in certain months or years (due to technical problems or stations not being active at the beginning of our study period). For each

weather station and each year, we obtained the monthly averages of daily rainfall (mm), and of daily maximum and minimum temperatures (°C). We further calculated the monthly averages between maximum and minimum temperatures for all the weather stations and years when data were available. Each weather station was classified as “coastal” or “inland” depending on its location, using the same criteria as for nests. We attributed to each nest the weather data from the nearest weather station located within the same region. An exception was, however, made for 18 nests located inland, but for which the corresponding closest weather station was located 230 km away: for these we instead used the closest “coastal” weather station, as the distance between these nests and this weather station was relatively small (i.e. between 35 and 70 km away), and at a similar altitude. On the other hand, we excluded weather data entirely for 6 nests for which the distance between them and their closest weather station was further than 120 km. Overall, our sample size included 475 nesting events with associated weather data from weather stations that were located on average 29 ± 22 (SD) km away from study nests (Figure 8).

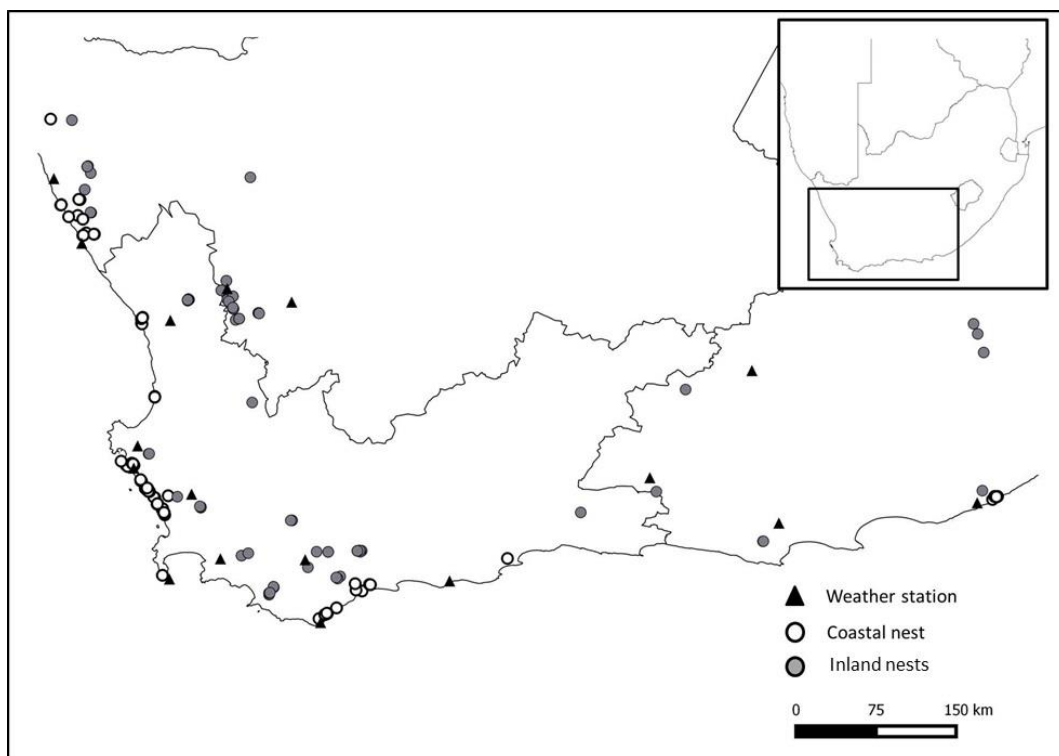


Figure 8. Location of study nests within South Africa for which weather data were available ($n = 475$), during 2000-2014. White circles: coastal nests; dark grey circles: inland nests; black triangles: weather stations that provided data for the purpose of the study.

Statistical analyses

Weather, timing of breeding and breeding parameter analyses

All statistical analyses were conducted using R 3.2.3 (the R Foundation for statistical computing 2015).

To reduce the number of weather variables and to account for potential collinearity among them, we conducted a Principal Component Analysis (PCA) on monthly rainfall and temperature data for each station and study year. We selected the first four weather Principal Components (PCs) for subsequent analyses, i.e. a scree plot showed a marked drop in explained variance between the fourth and the fifth PC. PCs were chosen for analyses on the effect of weather on breeding parameters, rather than using raw weather data, because we did not have a strong *a priori* hypothesis of the time period over which weather may be more influential. Therefore, using raw data would have meant exploring the effect of a high number of potential explanatory variables, i.e. weather conditions over different time periods. Furthermore, our PCs had clear biological meanings (see results), which helped in interpreting the relationships found. However, because PCs include information about weather in all months, in our discussion we place most emphasis on the meaning of each PC for the months prior to the variable in question; for example in the relationship between lay date and weather, we focus on the meaning of each PC for the months prior to laying, not subsequently.

We investigated regional differences in the weather PCs using General Linear Mixed Models (GLMMs, statistical package lme4; Bates et al. 2012) that included the weather station identity as a random effect (to take into account the non-independence of the data coming from the same station) and the factors region (coastal vs. inland) and years (14 levels) as explanatory variables. This analysis was run on a data set that had only one data point for each weather station and year (n= 88).

To analyse factors affecting variation in breeding phenology, we used GLMMs that included year as a random effect, so that we could identify patterns that would describe what happens in an average year. The “lay date” of each nest (response variable) was fitted with a Gaussian distribution and an identity link function. The initial model included the explanatory variables of region and weather variables (the first four weather PCs). These

models were conducted on a subsample of 393 nests for which both lay date and weather data were available.

GLMMs with year as a random effect were also used to explore clutch size, nesting success and productivity (response variables) in relation to region, lay date, and weather (explanatory variables). Initial models also included the interaction between region and lay date to look for regional differences in seasonal variations in breeding performance. For models where this interaction was significant, we re-ran the same model but without the interaction to test for differences between regions. Nesting success was fitted with a binomial distribution, and clutch size and productivity were fitted with a Gaussian distribution. While the latter may not be ideal for productivity data, using a Poisson distribution produced models with large dispersion parameters, whereas Gaussian models performed well and model residuals were normally distributed. Analyses of clutch size were conducted on a subsample of 183 breeding events for which clutch size, lay date and weather data were available. Analyses of variation in nesting success and productivity were conducted on a subsample of 223 and 222 breeding events, respectively, for which lay date and weather data were also available.

A stepwise backward procedure was performed for model selection (with the function `drop1`), and likelihood ratio χ^2 tests (LRT) on AIC differences were used to select the best models.

Bootstrap analyses

Sample sizes differed between regions and our slope estimates for the relationships between lay date and breeding parameters could be influenced by this or hinge on data from a few very early or very late nests (see Figure 10). In order to be confident that regional differences were not simply a consequence of these potential biases, we randomly selected a reduced and equal number of nests in each region and re-estimated the slope of the relationships and their 95% confidence intervals using a bootstrap analysis implemented in R 3.2.3. For the relationships between lay date and clutch size, our sample sizes included 144 and 42 nests in coastal and inland regions, respectively, so we re-estimated the slope using 1,000 random samplings of 30 nests from each region. For the relationships between

lay date and productivity, our sample sizes included 163 and 64 nests in coastal and inland regions, respectively, and we re-estimated the slope using 1,000 random samplings of 50 nests from each region.

RESULTS

Weather: seasonality and regional differences

Study regions were characterized by different weather conditions (Figure 9a, b). Both regions experienced higher temperatures and less rain during summer months (December-March) than in winter months (May-September). However, temperature differences between summer and winter were more pronounced inland than along the coast. Additionally, coefficients of variation for both temperatures and rainfall were greater from April to October in inland than in coastal regions, indicating that weather conditions inland, at that time, were more variable in space (among nest localities) or time (years) than those in coastal regions. Rainfall levels halved between August and September in both regions, coinciding with the peak of lay date in Black Harriers (Figure 9b, c).

The PCA analysis on monthly rainfall and temperature data rendered four PCs explaining approximately 60% of the variance (Table 1). PC1 was positively related to temperature during all months, although the relationship was less marked during the winter months (June-August) when temperatures were overall lower (Figure 9a). PC2 was positively related to rainfall during all months, although the relationship was less marked during the summer and early autumn months (December-March), when rainfall levels were generally lower (Figure 9b). PC3 contrasted high temperatures in summer and early autumn (December-March) but were low in late autumn and winter (May-August), with lower temperatures in summer and higher in autumn-winter. Therefore, this PC refers to temperature seasonality. Finally, PC4 identified situations with higher rainfall in summer and early autumn (December-March), but lower rainfall in late autumn and winter months (May-August), thus reflecting rainfall seasonality.

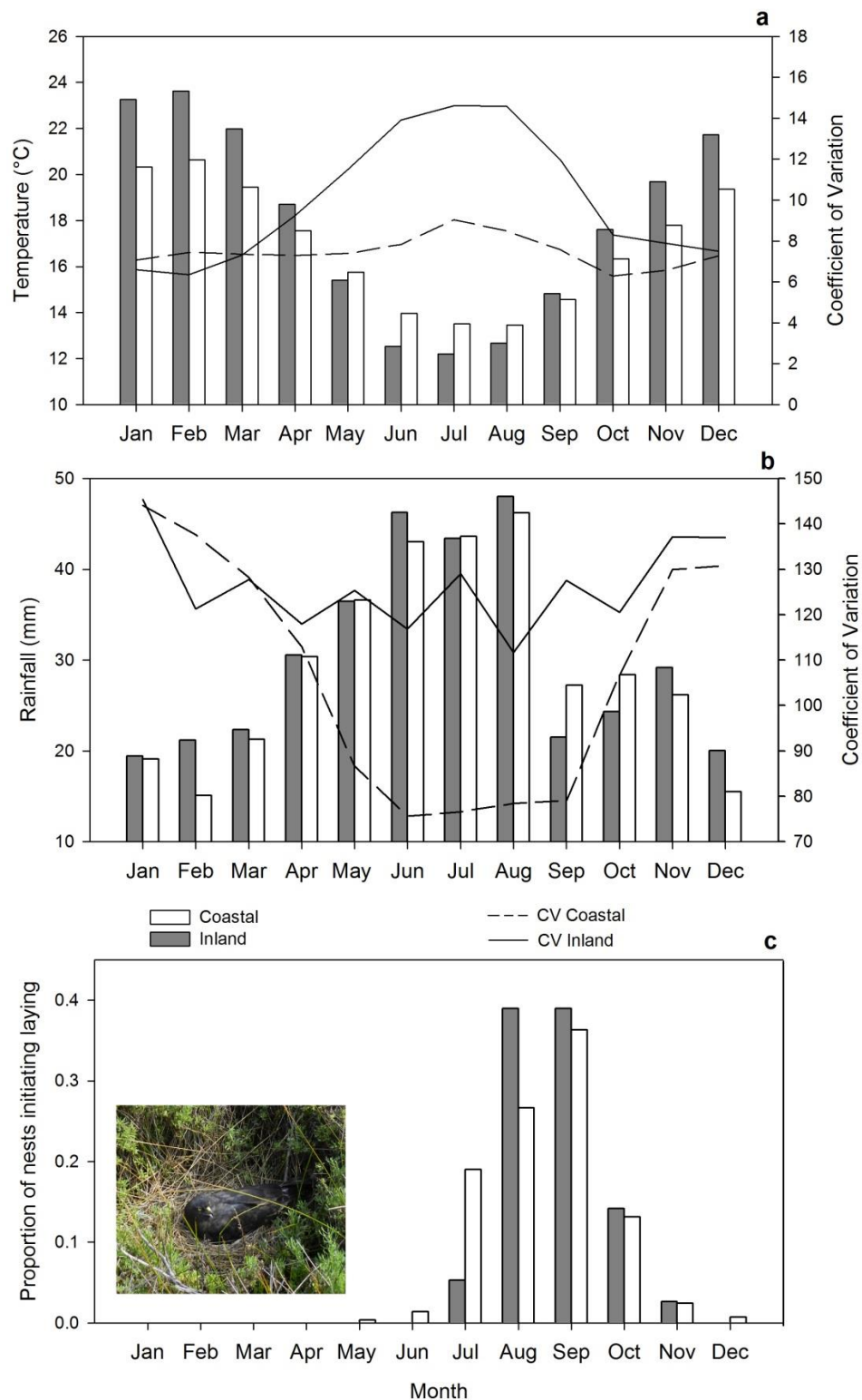


Figure 9. Monthly average temperature (a) and rainfall (b), according to region (coastal, white bars; inland, dark grey bars). Also presented are Coefficient of Variation ($100 \times \text{SD}/\text{Mean}$) for both climatic variables (dashed line for coastal, solid line for inland), as well as frequency distribution of breeding initiation ($n = 402$) (c) during the study period (2000-2014).

Table 1. Results of the Principal Component Analysis conducted on weather data (monthly averages of daily rainfall and daily temperatures) collected in 2000-2014 at 17 weather stations (see Figure 8). “Temp” represents the average of the daily maximum and minimum temperatures (°C), per month. “Rain” corresponds to the average of the daily rainfall (mm) per month. Variable loadings greater than 0.2 or lower than -0.2 are highlighted in bold. The months during which Black Harriers usually breed are highlighted in grey.

	PC1	PC2	PC3	PC4
Rain. JAN	0.014443	0.129292	-0.01123	0.353204
Rain. FEB	-0.02877	0.171029	0.098396	0.416038
Rain. MAR	-0.01663	0.196125	0.032239	0.45707
Rain. APR	-0.06009	0.303045	0.03444	0.067087
Rain. MAY	-0.11894	0.281114	0.093557	-0.23835
Rain. JUN	-0.12415	0.308008	0.131795	-0.26365
Rain. JUL	-0.124	0.302469	0.084539	-0.23318
Rain. AUG	-0.13295	0.325751	0.102831	-0.27611
Rain. SEP	-0.09693	0.350088	-0.01698	-0.04674
Rain. OCT	-0.0634	0.28149	0.001479	0.105491
Rain. NOV	-0.07844	0.32201	0.029495	0.174901
Rain. DEC	0.006074	0.168862	0.060451	0.290475
Temp. JAN	0.292895	0.057873	0.310684	0.011043
Temp. FEB	0.315231	0.065366	0.257761	-0.01914
Temp. MAR	0.303027	0.066636	0.231843	-0.11742
Temp. APR	0.335735	0.082603	-0.02066	-0.20133
Temp. MAY	0.230807	0.16073	-0.29901	-0.07898
Temp. JUN	0.127701	0.149213	-0.44499	-0.0962
Temp. JUL	0.127055	0.120859	-0.43149	-0.10167
Temp. AUG	0.190731	0.153715	-0.36607	0.128207
Temp. SEP	0.272202	0.082607	-0.18223	0.075789
Temp. OCT	0.352615	0.052975	0.017441	0.024666
Temp. NOV	0.344244	0.035273	0.119129	-0.00367
Temp. DEC	0.290036	0.046253	0.258563	0.009631
Variance explained				
Proportion	0.254	0.1554	0.1361	0.08704
Cumulative	0.254	0.4084	0.5454	0.63249

All weather PCs varied significantly among years, but only PC1 and PC3 were significantly different between regions (Table 2). PC1 values were lower in the coastal region (Least-Squares Means (hereafter LS means): -1.15 ± 0.70), indicating cooler temperatures (particularly in springs, summers and autumns) than inland (LS means: 0.93 ± 0.64). PC3 values were also lower in coastal regions than inland (LS means: -1.43 ± 0.34 and 1.18 ± 0.31 , respectively), indicating that temperature variation throughout the year was more pronounced in inland regions.

Table 2. Results of the General Linear Mixed Models (GLMMs) testing for differences between years and regions (coastal vs. inland) in weather variables (PC1, PC2, PC3, and PC4; see Table 1). The “weather station” identity was included as a random effect to take into account for the non-independence of data from the same locality. d.f.= degree of freedom, LRT= Likelihood Ratio Test.

Dependent Variables	Explanatory Variables	d.f.	LRT	P
PC1	Year	15	82.15	<0.0001
	Region	1	4.67	0.031
PC2	Year	15	32.11	0.006
	Region	1	0.02	0.88
PC3	Year	15	214.52	<0.0001
	Region	1	21.11	<0.0001
PC4	Year	15	95.19	<0.0001
	Region	1	0.04	0.83

Timing of breeding

Lay date (n= 393 nests) was remarkably spread through the year, spanning 8 months, from mid-May to mid-December, and followed a unimodal distribution in each region (Shapiro normality test, w= 0.98, p< 0.0001, n= 287 for the coastal region; w= 0.95, p= 0.0009, n= 106 for the inland region) with a peak during mid-August to end of September (Figure 9c).

Lay date was negatively associated with weather PC2 (slope: -0.26 ± 0.07) and PC4 (slope: -0.27 ± 0.10 ; Table 3), indicating that laying occurred relatively earlier under rainier conditions, particularly when rain was more intense in autumn, winter and spring, and when

summers preceding laying, i.e. 6 months before laying, were wetter. Lay date was also significantly different between regions (Table 3): nests located in coastal regions had overall earlier lay dates (LS means: 8.40 ± 0.22 , 15-30 August) than those located in inland regions (LS means: 9.36 ± 0.28 , 1-15 September). Individuals in coastal regions also laid over a more extended period, with breeding events in this region occurring up to two months earlier and one month later than in inland regions (Figure 9c).

Table 3. Results of the Generalized Linear Mixed Models (GLMMs) testing for variations in lay date (15-day periods), clutch size, nesting success and productivity. “Year” was included as a random effect in all models. Initial models included region (coastal vs. inland), weather variables (PCs) and lay date (for clutch size, nesting success and productivity), as well as interactions between region and lay date. Stepwise backward model selection was performed based on AIC values. We present results for final models. d.f.= degree of freedom, LRT= Likelihood Ratio Test.

Dependent Variables	Explanatory Variables	d.f.	LRT	P
Lay date				
	PC2	1	15.00	0.0001
	PC4	1	7.28	0.007
	Region	1	16.14	<0.0001
Clutch size				
	PC2	1	4.23	0.039
	Region×Lay date	1	7.45	0.006
Nesting success				
	Lay Date	1	17.59	<0.0001
Productivity				
	PC2	1	5.08	0.024
	Region×Lay Date	1	2.84	0.092

Breeding parameters

Clutch size averaged 3.58 ± 0.64 eggs (range: 2-5; n= 183 nests). Clutch size varied with rainfall (PC2, Table 3), with larger clutches being associated with wetter conditions (slope: 0.08 ± 0.04), particularly in autumn, winter and spring. Clutch size also varied significantly with the interaction between lay date and region (Table 3): clutch size declined markedly as the season progressed, but this decline was more pronounced inland (slope: -0.25 ± 0.07)

than in the coastal region (-0.05 ± 0.03) (Figure 10a). Bootstrapping analyses using 1,000 random samplings of 30 nests from each region indicated very little overlap between the estimates of slopes for each region (95 % confidence intervals of -0.17 to 0.05 for coastal nests and of -0.39 to -0.15 for inland nests). On removing the interaction region \times lay date from the model, clutch sizes were not significantly different between regions (LRT= 0.37, $p= 0.54$).

In total, 31 % of nests ($n= 223$) monitored during the study period failed to produce fledglings. Nesting success declined significantly with lay date (Table 3; slope: $- 0.40 \pm 0.10$), and this decline was similar between regions (the interaction region \times lay date was non-significant; Figure 10b). On controlling for lay date, no significant differences in nesting success were found between regions, nor any relationships between nesting success and weather variables (Table 3).

Productivity among monitored nests averaged 1.66 ± 1.30 fledglings (range 0-4 fledglings, $n= 222$ nests). Productivity was positively associated with weather PC2 (Table 3; slope: 0.12 ± 0.05), indicating that productivity increased in wetter conditions. Productivity also declined as lay date increased (Table 3; Figure 10c). As for clutch size, there was an indication that this seasonal decline in productivity differed between study regions (marginally significant region \times lay date interaction; Table 3; $p= 0.09$), with a steeper decline inland (slope: $- 0.40 \pm 0.12$) than in the coastal region (slope: -0.20 ± 0.05 ; Figure 10c). Bootstrapping analyses using 1,000 random samplings of 50 nests from each region showed that there was some overlap between the estimates of the calculated slopes for each region (Mean, SD, and 95% confidence intervals: -0.20 ± 0.05 , -0.30 to -0.08 for coastal nests; and $- 0.35 \pm 0.14$, -0.59 to -0.12 for inland nests). When removing the interaction region \times lay date from the model, there was no significant difference in productivity between regions (LRT= 0.002, $p= 0.98$). Nonetheless, when further comparing the overall production of Black Harrier fledglings (i.e. number of nestlings that successfully fledged) between regions and over the study period 2000-2014, results suggested that an average 158 nestlings successfully fledged in coastal regions vs. 53 inland. This was probably promoted by the overall higher number of active nests in coastal regions than inland (121 vs. 48, respectively).

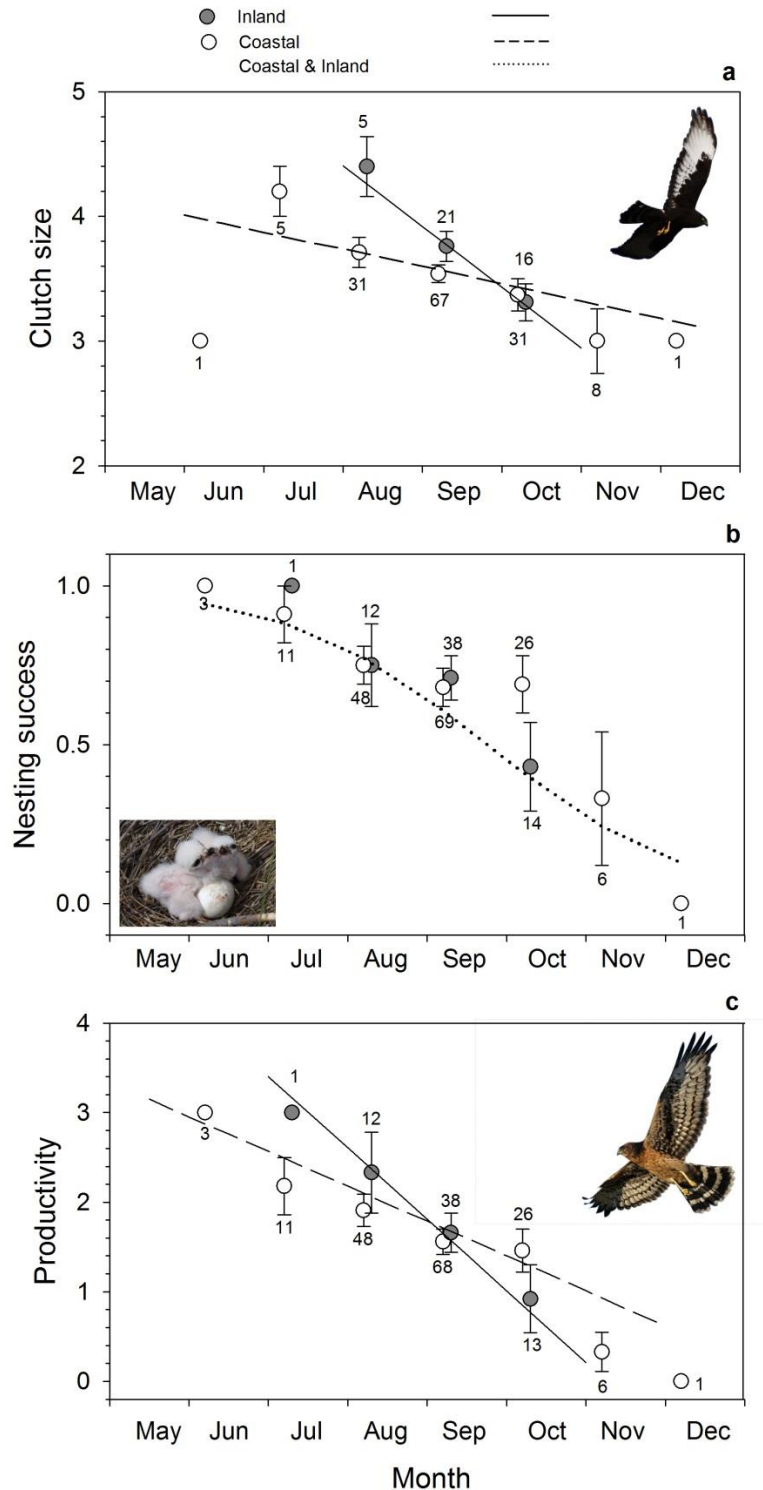


Figure 10. Black Harrier breeding performance **a**: clutch size; **b**: nesting success; **c**: productivity (i.e. number of fledged young) variation according to lay date and region (coastal nests: white circles/dashed line; inland nests: grey dark circles/ solid line). Lines represent modelled data from the GLMM results (Table 3). Raw data (circles) are also shown for illustration purposes and have been averaged over two consecutive 15-days periods. Sample sizes (number of nests) are indicated above the error bars. The point line represents the breeding success variation with laying date for both coastal and inland regions (this relationship did not differ between regions).

DISCUSSION

This study revealed an extended breeding period for the Black Harrier and profound consequences of the timing of breeding on breeding performance. Moreover, it is one of the few studies that document a seasonal decline in breeding performance in a southern African species (Simmons 2000; Martin et al. 2014; Murgatroyd et al. 2016b) and one of the few studies overall to highlight a regional difference in the strength of this seasonal decline. Seasonal declines in breeding performance appeared more pronounced inland, characterized by more seasonally variable weather conditions, than in coastal regions. These observations may explain why coastal regions are more frequently used by this scarce raptor and this has conservation implications, which we develop below.

Seasonal declines in breeding performance

Most strikingly, we found that seasonal declines varied among regions for clutch size and also (less markedly) for productivity. The seasonal decline in these parameters was progressive and moderate in coastal regions but much more abrupt in inland regions. Thus, clutch size and productivity were overall higher inland than in coastal regions early in the season, until September, but differences were not found or values were higher in coastal regions for nests initiated from October onwards. Interestingly, we did not find a significant difference between regions for nesting success, suggesting that regional differences in declines in productivity may simply result from differences in clutch size patterns. Additionally, this suggests that differences between regions are more influential early in the breeding cycle. Ultimately, neither clutch size nor productivity were, on average, significantly different between regions, indicating that differences between regions early and late in the season balanced each other out.

Seasonal declines in breeding performance can be explained by differences in the quality of individuals breeding early or late and/or by a worsening of environmental conditions as the breeding season progresses (Verhulst et al. 1995; Verhulst & Nilsson 2008). The overall seasonal decline observed in the Black Harrier population may reflect a difference in the quality of individuals breeding earlier vs. later in the season, with for instance older and more experienced birds breeding earlier in the season. However, the

observed regional differences in the seasonal declines are unlikely to be explained by differences in individual quality alone, particularly for a mobile species like the Black Harrier: evidence from satellite tagged birds indicate that the same individual can breed in both the coastal and the inland region in different years (Taylor 2015; RES *unpublished data*). Our results thus indicate that changes in environmental conditions likely play an important role in explaining seasonal changes in breeding performance, and furthermore that this degradation in environmental condition is stronger inland than in coastal regions.

Temperatures were overall higher in coastal than inland regions until August, coincident with smaller clutches at the coast. The opposite pattern was found from October onwards, when clutch sizes were greater in coastal regions coincident with relatively lower temperatures there than inland, and in this context temperature variation could be an indicator of the temporal variation in quality of environmental conditions among regions. However, temperature (PC1) did not significantly influence clutch size (or any other breeding performance parameter), so regional differences were likely to be better explained by other factors other than weather condition only, such as habitat quality and/or food availability, the latter of which may also be in turn, modulated by weather. Black Harriers has been described as mostly feeding on small mammals (ca. 65 % of the diet), particularly on Four-Striped Mouse *Rhabdomys pumilio* (hereafter Striped Mouse) and African Vlei Rats (*Otomys sp.*) (Van der Merwe 1981; Steyn 1982), so the smaller clutch size in inland regions for pairs starting to lay late in the season may reflect lower small mammal availability there at that time. Population dynamics and breeding output of the Striped Mouse are known to vary strongly with rainfall (see Taylor & Green 1976; Meynard et al. 2012; Rymer et al. 2013). This, together with our results (relationship between PC2 and both clutch size and productivity), suggests that greater rainfall during autumn and winter positively influence the abundance of small mammals such as the Striped Mouse. This may in turn influence breeding performance in Black Harriers, as found for other species (Korpimäki 1992; Salamolard et al. 2000; Redpath et al. 2002b). Future studies should investigate the relationship between Black Harrier's breeding and food availability, and how this varies in space and time.

Factors affecting lay date variations

Black Harriers showed a remarkably extended breeding period, with the onset of laying spread over 8 months (mid-May to mid-December). A wide spread in timing of breeding has been reported in other raptors from the Southern Hemisphere (e.g. 8 months for the Black Sparrowhawk *Accipiter melanoleucus*, Martin et al. 2014), including other harrier species, such as the African Marsh Harrier *Circus ranivorus* (9 months, Simmons 2000; Simmons et al. 2005), the Cinereous Harrier *Circus cinereus* (7 months, del Hoyo et al. 1994) or the Spotted Harrier *Circus assimilis* (5 months, del Hoyo et al. 1994). This contrasts to what is usually observed in harrier species breeding in the Palearctic, for which the timing of breeding rarely exceeds 3 months (e.g. Pallid Harrier *Circus macrourus*, Montagu's Harrier *Circus pygargus*, Hen/Northern Harrier *Circus cyaneus/hudsonius*, Schipper 1979; Simmons et al. 1986; Arroyo et al. 1998; Simmons 2000; Amar & Redpath 2005; Terraube et al. 2009). These large scale differences in the extent of the timing of breeding are likely related to climate. Indeed, the Northern Hemisphere is in general characterized by a more pronounced seasonality in rainfall and temperature regimes (García & Arroyo 2001; Redpath et al. 2002a) than the Southern Hemisphere, which may limit in time the conditions that are suitable for successful breeding. This, also suggest that conditions for breeding in the Southern Hemisphere are suitable over a longer time for Black Harriers, as for other African raptors.

Nevertheless, we found a clear seasonal peak, with most laying (ca. 50 % of clutches) occurring between mid-August and the end of September. This, together with the strong seasonal decline in breeding performance observed, indicates that optimal timing for breeding is limited for this species, despite the extended breeding period. This peak coincides with a sharp drop in rainfall levels and an increase in temperature, suggesting that high rainfall levels may impair laying. However, models showed that, overall, laying occurred earlier under more rainy conditions (negative relationships with PC2 and PC4), and particularly if rainfall was greater in autumn-spring periods and when summers preceding laying were wetter. The latter may reflect the east-west rainfall conditions in South Africa, as laying is earlier in eastern locations, where rainfall levels are overall higher particularly in winter months. This may also be associated with food availability: wetter summers may contribute to a greater primary productivity and subsequent rains just before laying (April-

May) and during breeding may lead to greater food abundance during Black Harrier's whole breeding cycle (see above).

The strong associations between timing of breeding, rainfall and temperature to a latter extent, also indicate that climate change may further influence shifts in breeding phenology of southern African birds (Simmons et al. 2004; Cunningham et al. 2013), including raptors (Martin et al. 2014), most notably in the southern and western regions where a warming trend has been detected during the past 50 years, and rainfall is predicted to decline (Hockey et al. 2011; Cunningham et al. 2015). Our results highlight that weather conditions, and most notably rainfall regime, play an important role in determining the timing of breeding of Black Harriers, and likely shapes the regional differences encountered in lay date. However, the timing of breeding may also depend on the seasonal fluctuation of other variables responsive to environmental cues that change with weather conditions, but that were not tested here. These more likely would be either variations in food supply (Perrins 1970; Verhulst & Nilsson 2008) or arrival dates from the non-breeding grounds, as suggested for other raptor species (Newton 1998; Ketterson et al. 2015).

We also found differences in lay date between regions: Black Harriers breeding in coastal regions started laying on average about 15 days earlier, and clutches occurred over a more extended period than those breeding inland (Figure 9c). These patterns suggest that optimal conditions for breeding might be achieved at different times in different geographical zones, but also indicate that suitable conditions for breeding may last longer in coastal regions than inland.

Conservation implications

Black Harriers have been described as Fynbos specialists (Curtis et al. 2004), due to a greater number of breeding events in this vegetation type along the coast and a higher productivity in coastal regions compared with inland regions. However, our results, based on a larger sample size over a longer study period, differ from those of that study: while the number of monitored breeding events was indeed larger along the coast, we found that overall productivity was equal in inland and coastal regions. This was mainly explained by the regional differences in seasonal decline observed in breeding parameters, with clutch size

and productivity being greater inland early in the season, but the subsequent decline being more abrupt. Environmental conditions inland might be more suitable than in coastal regions, but only for a limited period of about 1.5 months. Before that, they seem to be unsuitable to allow breeding, and thereafter, they quickly deteriorate inducing a reduced breeding performance later in the season. Weather conditions at the beginning of the harrier breeding season (until October) also appeared much more variable among sites and years in inland regions (greater coefficient of variation; Figure 9a, b). This implies that, even if average conditions are better at that time, in certain years or regions, conditions may not be suitable for breeding. In coastal regions, environmental conditions remain more stable throughout the harrier breeding season, which allows productive breeding to occur over a longer period of time (4 months) (Figure 9c). Thus, the more stable weather in coastal regions within and among years may mean that it is overall a safer choice for Black Harriers to breed there than inland. This may explain why breeding Black Harriers were more commonly found at the coast (i.e. 3 times more breeding events along the coast than inland; Curtis et al. 2004), although we recognise that these figures do not control for search effort.

Recent changes in climate conditions within Africa during the last decades (Hulme et al. 2001; Hockey et al. 2011; Kruger & Sekele 2012; Cunningham et al. 2015) may exacerbate the differences among regions and present a challenge for species like the Black Harrier. Indeed, a shift in rainfall and temperature patterns has occurred in South Africa and most notably in the south-west of the country, where most harriers breed: temperatures are getting warmer with less rain falling inland, the same pattern is expected in the western part of the Northern Cape Province, where many “inland” Black Harrier breeding events occur, while the opposite trend is expected along the coast (Cunningham et al. 2015). In addition, anthropogenic modifications in land use during the last century in South Africa such as the conversion of the Fynbos vegetation into agriculture or urbanization, might also negatively affect the Black Harrier population (Curtis et al. 2004). Only two records have mentioned Black Harriers breeding in cultivated areas (Steyn 1982; Chadwick 1997), which suggests that the species might not be capable of adapting to breed in non-natural habitats (Curtis et al. 2004; Jenkins et al. 2013), contrary to Palearctic Harriers (Arroyo et al. 2002; Millon et al. 2002) and other south African raptors (Murgatroyd et al. 2016b). Further land use change may “force” even more Black Harriers to breed along the coast, in Fynbos vegetation, where

environmental conditions remain more stable within and among years in comparison to other available sites (e.g., Karoo biome in the inland regions) that may become drier and warmer. Additionally, and considering that coastal breeders can initiate clutches for longer (i.e. 8 months) than inland breeders (i.e. 5 months), and that accordingly more nests were found along the coast than inland, overall and in absolute numbers, coastal breeders probably add more to future generations than do those breeding inland, even if average productivity is similar among regions. Indeed, estimates of total number of fledglings produced in coastal areas over the study period almost tripled that of fledglings produced inland. All this highlights the importance that the coastal Fynbos has for the stability and sustainability of the Black Harrier population in the future. Conservation measures have already prioritized the protection of Fynbos vegetation, through the creation of national parks and private reserves, and should continue in order to conserve the species in the long term.

CONCLUSIONS

This study provides evidence for spatial variation in the strength of seasonal declines in breeding performance, something that has only received scarce support previously. This main finding has broad implications for population biology and conservation. Environmental heterogeneity needs to be accounted for when considering overall population viability, and our findings suggest that where environmental conditions are less variable and more predictable, the timing of breeding may have less importance for the production of young. Relative differences in individual quality between early and late breeders, which can explain the breeding seasonal declines (Verhulst & Nilsson 2008) would also potentially matter less. These areas may therefore constitute population sources and play a key role for overall population viability. In areas where seasonal declines are more pronounced, a mistiming of breeding will reduce offspring production and populations will be less buffered from rapid, unpredictable environmental changes. Studying spatial variations in the strength of seasonal productivity declines, as we did with the scarce Black Harrier, could help identify important breeding areas for long-term population viability.

Appendix A. Aging nestling Black Harriers using growing curves based on biometric measurements.

When conducting studies on wild animals, it is useful to accurately age the sampled birds (Clark 2007). This is even more important when working with nestlings, as their metabolism, physical and physiological condition tend to change rapidly from new born until fledging (Blem 2007). For harrier nestlings, the use of growing curves, based on various biometrical measurements, is often used to determine a nestling's age (e.g. Brignon 1997; Arroyo 1999). The construction of growth curves requires taking biometric measurements of known age individuals at different times, i.e. from its hatch throughout its growth until fledging, something that is very time-demanding and challenging when working with wild species.

Methods

Nestling Black Harrier's hatching date was determined either directly when a clutch was found with an egg hatching, or from a newly hatched chicks, estimated to be 1-3 days old, with a body mass similar to the average egg weight. As just-hatched nestlings cannot be ringed because of their small body size, to identify each nestling at the next visit, we marked the down on their crown using a natural and non-toxic waterproof stain colouration (green, violet or black) (Kiki, pintura especial, www.gzmsl.com). This product is often used by raptor veterinaries in rehabilitation centres, and has been shown to have no negative effects for individuals. When nestlings were 1-15 days old, their body mass was measured to the nearest 0.1 g using an electronic balance (Figure A1), while a pesola to the nearest 5-10 g was used for older nestlings, i.e. which were put into a cotton bag (Figure A2). The wing and tail (only apparent after a few days of growth) lengths were measured to the nearest 0.5 cm using a ruler (Figure A3).

In total, 22 nestlings of known hatching date were sampled during 2013 (n= 2) and 2014 (n= 20) breeding seasons. Nest visitation frequency varied between 2 and 10 visits, i.e. 5.5 on average, and data were collected throughout nestling's growth, from 1 to 39 days old. From the 22 chicks, 124 biometric measurements were collected over their time in the nests. Nestlings were all sexed genetically using DNA analysis from blood samples (see Chapter 4). Three growth curves were constructed, for each sex separately: body mass in

relation to age (fitted curve sigmoid 4 parameters; Figure A4), wing length in relation to age (fitted curve segmented 2 linear; Figure A5) and tail length in relation to age (fitted curve segmented 2 linear; Figure A6). We used segmented regressions for tail and wing growth so that we could easily back-transform these measurements into an estimated age above a given threshold value using linear regression. We used R 3.2.3 (the R Foundation for statistical computing 2015) for analyses.

Results

For wing length, we could predict the age of nestlings older than 9 days: using the package “segment”, results indicated a cut-off point of the curve at 8.5 ± 0.5 days. The linear equation used to determine the age of nestlings with wing length measurement was: $\text{Age} = (0.13 \pm 0.002) \text{ Wing length} + (2.66 \pm 0.27)$. Similarly, for the tail length, we could predict the age of nestlings older than 13 days, where results indicated a cut-off point of the curve at 13.4 ± 0.5 days. The linear equation used to determine the age of nestlings with wing length measurement was: $\text{Age} = (0.14 \pm 0.005) \text{ Tail length} + (13.84 \pm 0.46)$. Of note is the finding that until the maximum age of sampled nestlings (39 days), the tail and wing growth curves do not differ between male and female nestlings and that the same equation can be used to predict a nestling ‘age, irrespective of its sex. Mean \pm SD tail length averaged 233.2 ± 5.9 for adult males and 255.9 ± 7.9 for adult females, and were significantly different from each other (t-test: $p < 0.0001$). Mean \pm SD wing length averaged 343.9 ± 4.65 for adult males and 369.3 ± 4.83 for adult females, and were significantly different from each other (t-test: $p < 0.0001$). Sexual size dimorphism in tail and wing length therefore appears after fledging (nestlings older >40 days), since males end up smaller than females as they mature. On average, nestling females leave the nest with 75.1% of their full wing feather growth and 63.1 % of their full tail feather growth; males leave the nest with 74.3% of their full wing feather growth and 67.7 % of their full tail feather growth.

Furthermore, we also conducted t-tests on the body mass between nestlings aged 35-39 days (start of the plateau, Figure A6) and the body mass of mature adults, calculated for each sex separately. Results showed no significant differences between male nestlings and the adult male body mass ($p = 0.15$), or for female nestlings and adult female body mass ($p = 0.98$). These results suggest that nestling Black Harriers leave the nest with a body mass

similar to the one reached at maturity. Of note is that all the adult females were captured when their nestlings were approximately 20 days old, so their body mass is likely to be similar the one that they would have outside the breeding season and therefore, the adult-nestling weight comparisons are unbiased by seasonal changes in body mass.



Figure A1. Black Harrier nestlings about 2-5 days old being weighed in the field using an electronic balance.

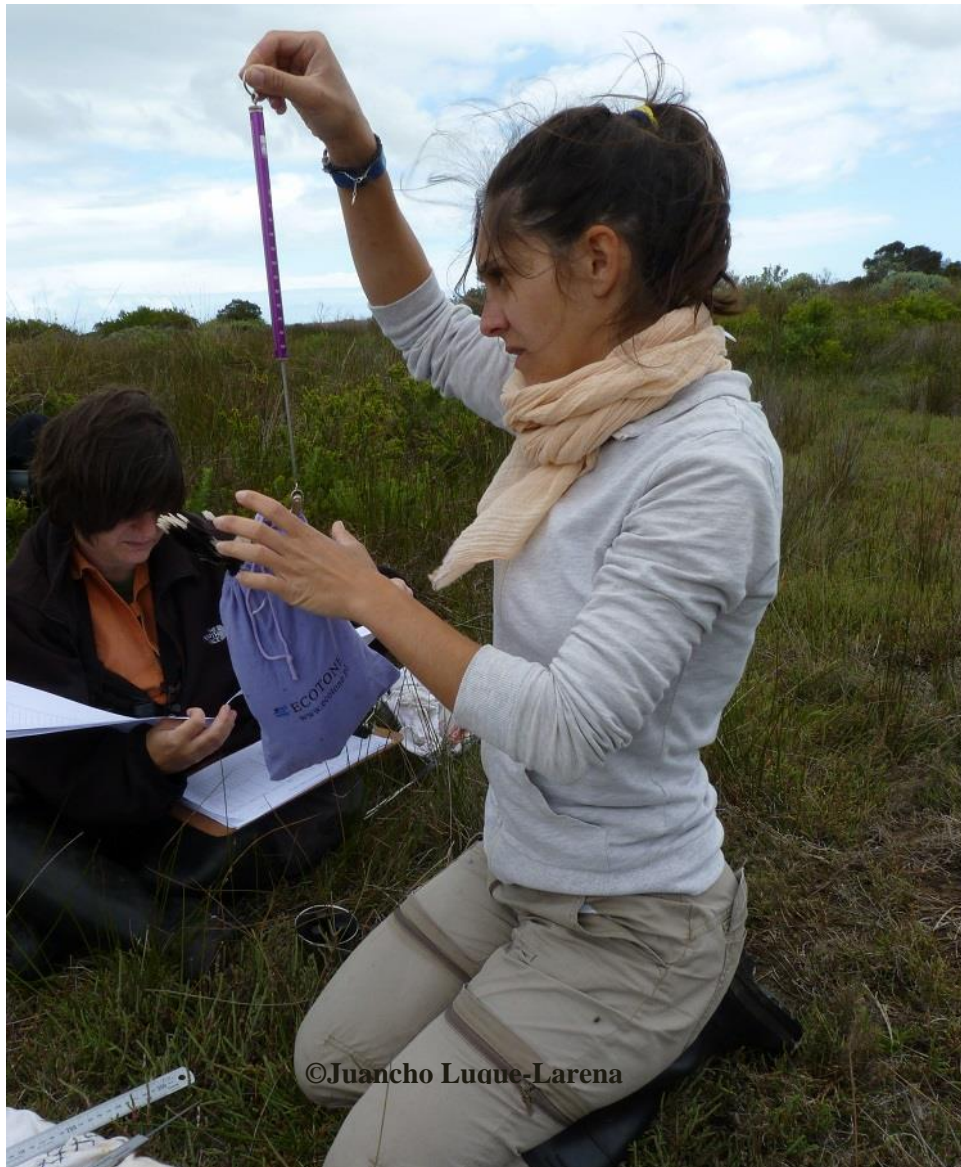


Figure A2. MSGH weighing a Black Harrier nestling older than 15 days at the West Coast National Park (coastal region). Nestlings aged 15-39 days were put into a cotton bag and weighed with a pesola.



Figure A3. Measurement of the tail of a Black Harrier nestling about 20 days old with a ruler. A soft cotton bag was gently deposited over the nestling's head to reduce potential stress caused by the biometric measurements.

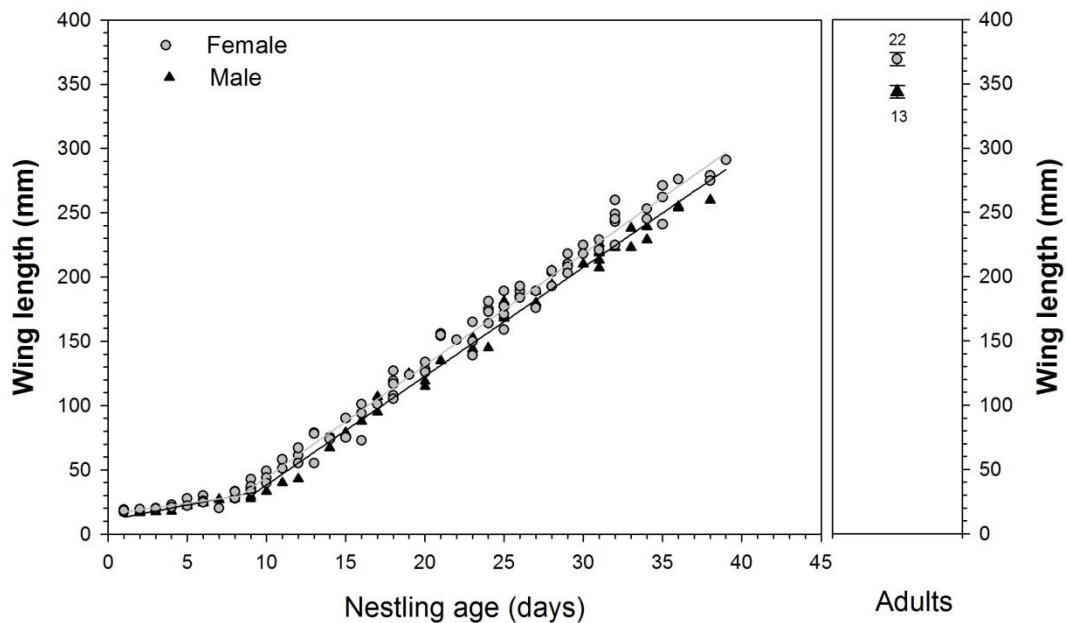


Figure A4. Wing length (mm) according to age and sex (female: grey circle/grey solid line; male: black triangle/black solid line) in nestlings ($n = 22$) aged 1-39 days and in adults ($n = 35$) (right). Here, a segmented 2 linear curve was fitted to the data.

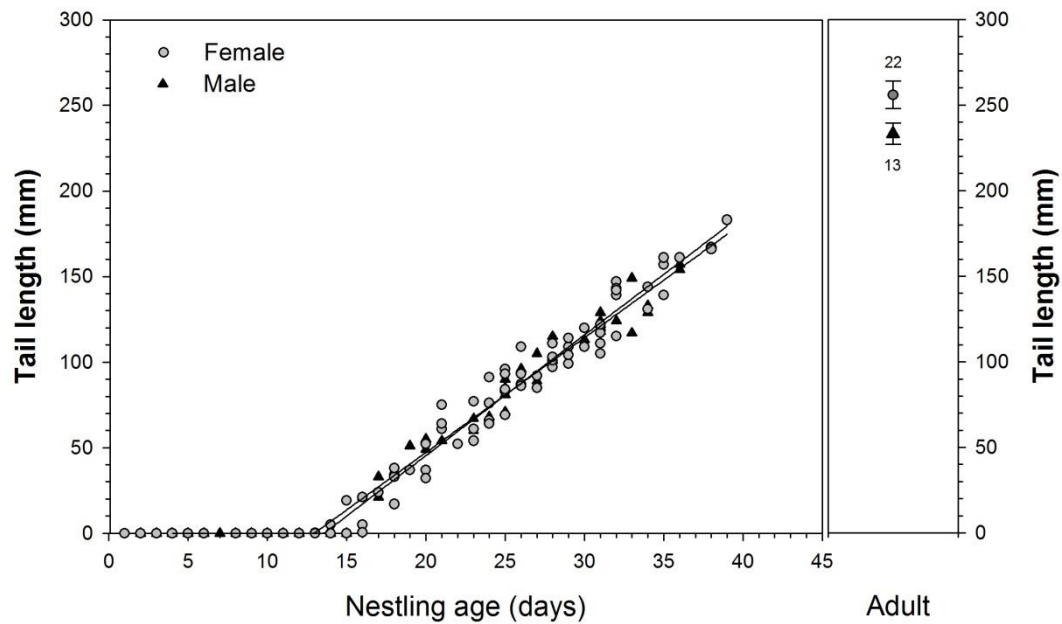


Figure A5. Tail length (mm) according to age and sex (female: grey circle/grey solid line; male: black triangle/black solid line) in nestlings ($n = 22$) aged 1-39 days (left) and in adults ($n = 35$) (right). Here, a segmented 2 linear curve was fitted to the data.

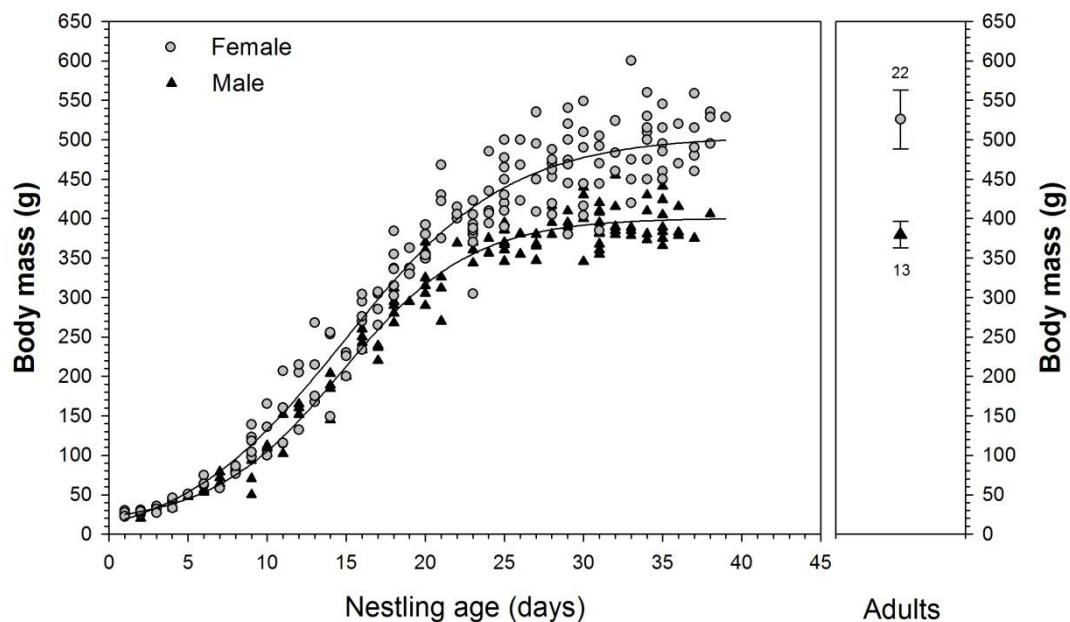


Figure A6. Body mass (g) according to age and sex (female: grey circle/grey solid line; male: black triangle/black solid line) in nestlings ($n = 93$) aged 1-39 days (left) and in adults ($n = 35$) (right). Here, a curve sigmoid 4 parameters was fitted to the data. For this figure, more data points appear in the graph because we used an estimated age from tail and wing (based on the results of Figures A4 and A5) to include those nestlings for which we had a value of body mass, but not its age ($n = 71$ nestlings and $n = 132$ biometric measurement).

Chapter 2

Is the Black Harrier *Circus maurus* a specialist predator? Assessing the diet of an Endangered raptor species endemic to southern Africa



A modified form of this chapter has been published in Ostrich, Journal of African Ornithology as: García-Heras M-S., Mougeot F., Arroyo B., Avery G., Avery M.D. & Simmons R.E. 2017b. Is the Black Harrier *Circus maurus* a specialist predator? Assessing the diet of a threatened raptor species endemic to southern Africa. *Ostrich: Journal of African Ornithology*, 2017: 1-8.

ABSTRACT

Studying the diet of wild animals is central for understanding their flexibility in food requirements. The Black Harrier *Circus maurus* is an Endangered raptor in South Africa, Namibia and Lesotho. To date, information about the diet of the species is insufficient for a comprehensive understanding of its ecology. We studied the diet composition of breeding Black Harriers using ca. 1,000 pellets (> 1,700 identified prey) collected at nest sites in two geographical regions (coastal vs. inland) over 10 breeding seasons (2006-2015). We show the importance of small mammals in Black Harrier diet (64.4 % and 78.2 % of prey and consumed biomass, respectively), with the Four-Striped Mouse *Rhabdomys pumilio* being a main trophic resource. We also reveal the importance of birds and reptiles as alternative prey, particularly in inland regions, and show inter-annual variations in diet in both regions. Our study confirms that this species can be considered a small mammal specialist. Specialist predators are more vulnerable than generalist ones and diet specialisation has been linked with a poorer conservation status in other species. Our results thus have implications for the conservation of this species in southern Africa. These are highlighted for the long-term sustainability of this Endangered endemic species.

INTRODUCTION

Understanding the feeding habits of animals and assessing food resource use is at the core of ecological research (Martínez del Río et al. 2009). Food supply is often the main factor affecting population densities of many bird species, including raptors (Newton 1979). The study of diet provides basic background information to understand food requirements, which is a crucial step in understanding any species' ecology (Arroyo 1997).

Specialist species use a narrow range of resources and preferentially feed on a given food type regardless of its abundance (see Pyke et al. 1977; Stephens et al. 2007), while generalists feed on a broader range of food types, varying opportunistically in response to changes in availability (Terraube & Arroyo 2011; Nadjafzadeh et al. 2016). However, there is often a continuum from specialization to generalization within and between species (Partridge & Green 1985; Woo et al. 2008). Typically, specialists are more efficient than generalists when foraging for their favoured prey, which is possibly associated with better adaptations or search images (MacArthur & Pianka 1966; Dukas & Kamil 2001; Terraube et al. 2010, 2014). However, the foraging success of specialists may be much lower than that of generalists when they target alternative prey (Terraube et al. 2010). In this context, a broader diet in specialist species may indicate a general decrease in the abundance or availability of the primary prey (Steenhof & Kochert 1988) that forces individuals to feed on alternatives and may be associated with a lower reproductive success (Korpimäki 1992; Bolnick et al. 2003; Arroyo & García 2006; but see Whitfield et al. 2009 and Murgatroyd et al. 2016a for contradictory results).

The Black Harrier *Circus maurus* is an endemic ground-nesting raptor of southern Africa. The population of this medium-sized bird has been estimated at less than 1,000 breeding individuals, and the species is listed as Endangered in South Africa, Namibia and Lesotho (Simmons et al. 2015; Taylor 2015), comprising its entire breeding range. Black Harriers breed in natural vegetation in both coastal and inland regions of south western South Africa (Curtis et al. 2004; Curtis 2005; Chapter 1). The species has been tentatively described as a small mammal specialist (Van der Merwe 1981; Steyn 1982), although quantitative information on its diet is scarce and inconclusive. A previous study based on a smaller sample size over three years (2000-2002) suggested that the diet may vary with

geographical location: individuals breeding along the coast appeared to feed primarily on small mammals, while those breeding inland had a more diverse diet, including more birds (Curtis et al. 2004). Nonetheless, detailed information about the diet of this Endangered species, and annual or regional variations, is overall lacking.

The aim of this study is to describe the diet composition of this scarce and Endangered species, to evaluate the numerical and biomass importance of the different categories of prey, and to examine potential regional or inter-annual variations in the diet. For this, we used a large data set of pellets (ca. 1,000; 1,760 identified prey) collected at active Black Harrier nests in South Africa during 2006-2015 across the breeding range.

MATERIALS AND METHODS

Study sites

This study presents diet information collected over 10 years (2006-2015 breeding seasons) in two Provinces of south western South Africa. Geographically this spans the coast of the Western Cape Province (33.700° S, 18.45° E; 33.133° S, 18.083° E), and inland in the Northern Cape Province in the Nieuwoudtville area (-31.316° S, 19.083° E). Nests were located in and around National Parks (South African National Parks – SANParks properties), Provincial Protected Reserves (Cape Nature), or on private lands, spanning two main biomes, the Succulent Karoo and Fynbos (see Mucina & Rutherford 2006; Manning 2007; Chapter 1 for details on vegetation types). Depending on specific geographical location characteristics (i.e. altitude, distance to the coast and biome), each nest was classified as coastal or inland. This classification was initiated by Curtis et al. (2004) to explore regional differences in lay dates and productivity in Black Harriers, and was also used and detailed in Chapter 1. Environmental conditions between the two regions (e.g. climate conditions, availability of prey) are also known to be different (see Mucina & Rutherford 2006; Manning 2007; Curtis et al. 2004). Coastal nests were defined as those within 15 km from the coast and with a maximal altitude of 100 mASL, and those nests located further than 15 km from the coast and with an altitude higher than 100 mASL were considered as inland (see Chapter

1 for more details). Black Harrier nests in inland regions were mostly within the Karoo biome, whereas those along the coast were within the Fynbos biome (see Chapter 1).

Pellet collection and analyses

The diet was assessed through analysis of regurgitated pellets containing prey remains such as bones, scales, feathers or hairs. Pellet analysis is a widely accepted technique for diet studies in raptors, including harrier (*Circus*) species (Errington 1930; Simmons et al. 1991; Arroyo 1997; Redpath et al. 2001). Some biases are inherent in analysing pellets, and sometimes other methods or a combination of methods are preferred depending on diet composition (Simmons et al. 1991; Redpath et al. 2001). However, since these biases are likely to be similar among areas and times of year, they allow within-species comparisons.

Active nests and perch sites (e.g. posts or bushes known to be used by a specific breeding pair) were regularly checked for pellets. Pellet collection was extended over the Black Harrier breeding season: August-December in the coastal region and September-December in the inland region (Chapter 1). A total of 954 pellets was collected from 119 breeding sites (660 pellets from 83 breeding sites in coastal regions, and 294 pellets from 36 in the inland regions). Sample sizes varied between regions and years (Table 4).

After collection, pellets were air-dried and then transferred to individually labelled sealed plastic bags until analysis. We identified prey to the lowest taxon level possible (i.e. to species level) using the comparative osteology collections of the Iziko South African Museum, in Cape Town, South Africa. We determined the minimum number of individuals per taxon in each pellet based on: 1) for small mammals, the highest number of left or right mandibles, or left or right maxillaries, number of incisors and skulls; 2) for birds, upper and lower bills, left or right tarsus or other items that indicates presence; 3) for reptiles, left and right mandibles, maxillaries, or limbs that indicates presence. Reptiles were divided into two size categories (large and medium-small) based on the size of the largest skin scales within a pellet (i.e. ≤ 2 mm for medium-small and ≥ 2 mm for large). Small numbers of insect remains ($n=75$) were present in some pellets, in the form of very small pieces of body parts (e.g. mandibles, legs, elytra). However, we suspect that these came from the stomach contents of the birds and/or reptiles also present in those pellets. Black Harriers have rarely been

described feeding on insects (Van der Merwe 1981; Steyn 1982; *authors personal observations*). Therefore, as insects are probably rarely voluntarily ingested by Black Harriers, and represented an insignificant amount of biomass, they were not considered in the diet analyses.

Table 4. Sample sizes (number of pellets analysed, their corresponding number of contributed prey items (in parentheses), and the corresponding number of nests [in square brackets]), for the Black Harrier diet analysis according to year and region (coastal and inland). Fresh pellets were collected at or near Black Harrier active nests during each breeding season (July-December).

Years	Coastal Pellets (prey item) - [number of nests]	Inland Pellets (prey item) - [number of nests]	Total Pellets (prey item)
2006	8 (18)-[2]	7 (16)-[6]	15 (34)
2007	24 (46)-[3]	-	24 (46)
2008	27 (41)-[6]	-	27 (41)
2009	29 (58)-[7]	24 (54)-[5]	53 (112)
2010	69 (116)-[15]	-	69 (116)
2011	5 (9)-[2]	16 (35)-[4]	21 (44)
2012	96 (163)-[15]	-	96 (163)
2013	98 (162)-[14]	100 (214)-[13]	198 (376)
2014	186 (347)-[15]	147 (244)-[10]	333 (591)
2015	118 (237)-[12]	-	118 (237)
Total	660 (1197)-[91]	294 (563)-[38]	954 (1760)

For pellets containing fur only, hair imprints were conducted to identify small mammal species or group species, where possible. The microstructure of hairs (i.e. specific cuticular scale patterns on the surface of the hair; Ryder 1973) is a useful tool for identifying mammals, and has been widely used to assess carnivore diets (Bothma & Le Riche 1994; Breuer 2005) and the diets of scavenger raptors such as vultures (Donazar et al. 2010). A thin coat of clear nail varnish was applied to a clean microscope slide, and left to dry for about 5-10 seconds. Then, ten randomly selected hair from the same pellet were placed on the nail polish and left to set over for 24 hours. The hairs were then removed with tweezers, being careful to not damage the imprints. Each hair shape was then analysed with a light microscope at 10x and 40x magnification, and compared to those illustrated in Keogh

(1975). This technique was applied to 105 pellets and resulted in the identification of 174 specimens to species or small mammal group.

Overall, 1760 prey items we identified at the group or species level, 1685 without counting the insects.

Biomass estimation

To assess the importance of prey categories in diets that include very diverse prey in relation to size, it is important to estimate their relative contribution in terms of biomass (Arroyo 1997). We therefore present the dietary results as both the percentage of identified prey and their estimated biomass. For prey identified to species level, we used the average weight described for the species in Stuart & Stuart (2015) as an estimate of biomass. For broader prey categories, we used an estimated average weight (Table 5). For the assignment of biomass for “unidentified birds”, we took into account that the identified birds consumed in inland regions included a higher proportion of Galliformes (particularly Common Quail *Coturnix coturnix*) than in coastal regions, where Passeriformes were more frequently consumed (see results, Table 5). We calculated what would be the average weight of an unidentified bird from the coast or from the inland regions taking into account the relative proportion of identified Passeriformes and Galliformes in each region. Hence, an “unidentified bird” found in the coastal region was attributed a weight of 45 g (since most identified birds here were Passeriformes), while “unidentified birds” from the inland regions were attributed a weight of 69 g, because most of the birds identified there were Galliformes (see Table 5).

Statistical analyses

We used R 3.2.3 (the R Foundation for statistical computing, 2015) for statistical analyses. Differences in diet composition (relative proportions of small mammals, birds and reptiles) between regions (coastal vs. inland) were investigated using Chi-square tests. Similarly, inter-annual variations in diet composition in a given region were also tested using Chi-square tests. For this latter test, we used only information from years for which we had a minimum of 25 identified prey. Thus, analyses for the coastal region excluded the years 2006 (n= 15, excluding insects) and 2011 (n= 7, excluding insects). Similarly, analyses for the

inland region were only conducted for the years 2009, 2011, 2013 and 2014, because sample size was too small for 2006 ($n=14$) and 2008 ($n=20$), and because no pellets were collected in 2007, 2010, 2012 and 2015 (see Table 4). Coefficients of Variation were calculated as $100 \times \text{Standard deviation}/\text{mean}$, and diet diversity was calculated using the Shannon Diversity Index (H') as $H' = -\sum P_i \log P_i$, where $P_i = X_i/X$, and X_i = number of prey items taken from class i ; X = total number of prey items.

RESULTS

Diet composition

Black Harriers preyed upon three main prey types: small mammals (64.4 % of 1,685 identified prey, excluding insects), birds (19.2 %) and reptiles (16.3 %) (Table 5).

Among the identified small mammals, Black Harriers fed primarily on the Four-Striped Mouse *Rhabdomys pumilio* (hereafter Striped Mouse) (72.5 % of 487 identified small mammals), whereas the second main group was formed by Otomyinae (*Myotomys unisulcatus* and/or *Myotomys irroratus*), and *Micaeamys namaquensis* (25.3 % of the identified small mammals; Table 5). Black Harriers also fed to a lesser degree (< 3.0 %) on Musk Shrews *Crocidura cyanea*, Forest Shrews *Myosorex varius*, and Elephant Shrews *Elephantulus* sp., Grant's Golden Moles *Eremitalpa granti* and Cape Dune Mole-Rats *Bathyergus suillus* (Table 5). However, more than half (55.2 %) of small mammal prey remained unidentified, because neither teeth nor jaws were found in the pellets, or because the shape of some hair imprints did not allow identification (Table 5). Overall, small mammals represented the main prey (78.2 % of consumed biomass).

Among birds, Passeriformes were the most common prey (73.5 % of 147 identified birds), followed by Galliformes (25.2 % ; mainly Common Quails *Coturnix coturnix*). The remainder 1.3 % were identified as Columbiformes (Table 5). In some cases, bones and feathers were too damaged to allow identification to species level, and an additional 176 (54.5 %) birds remained as "unidentified". Information from identified prey showed that, in the inland regions, Black Harriers fed on Galliformes and Passeriformes in equal proportions,

whereas in the coastal regions, they fed predominantly on Passeriformes (Table 5). Overall, birds represented the second main prey in terms of contributed biomass (16.6 %) (Table 5).

Table 5. Prey categories and species identified in Black Harrier pellets (n= 954) from the coastal regions (n= 660 pellets from 83 active nests) and from the inland regions (n= 294 pellets from 36 active nests). Pellets were collected during the 2006-2015 breeding seasons. A weight (in g) is also given for each prey category as an estimate of prey unit biomass; this is the average weight given for the species. When the prey was unidentified to genus or species level, an estimated weight (ψ) is given (see Materials and methods).

	Prey weight (g)	Coastal N prey (% Biomass*)	Inland N prey (% Biomass*)
Small mammals			
<i>Bathergus suillus</i>	750	1 (1.08)	-
<i>Crociodura cyanea</i>	9	1 (0.01)	-
<i>Elephantulus sp.</i>	46	1 (0.07)	4 (0.56)
<i>Eremitalpa granti</i>	23	2 (0.07)	-
<i>Micaelamys namaquensis</i>	50	-	1 (0.15)
<i>Myosorex varius</i>	14	2 (0.04)	-
<i>Myotomys unisulcatus</i>	125	3 (0.54)	8 (3.04)
<i>Rhabdomys pumilio</i>	50	255 (18.40)	98 (14.92)
Otomyinae ψ	150	95 (20.56)	16 (7.31)
Unidentified ψ	72	416 (43.21)	182 (39.90)
Total small mammals		776 (83.98)	309 (65.88)
Birds			
<i>Galerida magnirostris</i>	45	1 (0.06)	-
<i>Coturnix coturnix</i>	95	4 (0.55)	33 (9.54)
<i>Crithagra flaviventris</i>	18	1 (0.03)	-
<i>Estrilda astrild</i>	9	1 (0.01)	-
<i>Ploceus capensis</i>	44	1 (0.06)	-
<i>Ploceus velatus</i>	34	2 (0.10)	3 (0.31)
<i>Pycnonotus capensis</i>	39	3 (0.17)	-
<i>Streptopelia senegalensis</i>	81	1 (0.12)	1 (0.25)
Alaudidae ψ	45	2 (0.13)	1 (0.14)
Coliidae ψ	51	2 (0.15)	-
Fringillidae ψ	20	4 (0.12)	-
Ploceidae ψ	40	1 (0.06)	2 (0.24)
Pycnonotidae ψ	40	3 (0.17)	-
Sylviidae ψ	20	1 (0.03)	-
Passeriforme (medium size) ψ	40	36 (2.08)	16 (1.95)
Passeriforme (small size) ψ	20	16 (0.46)	12 (0.73)
Unidentified	45 and 69 ϕ	101 (6.56)	75 (15.76)
Total birds		180 (10.85)	143 (28.92)

Table 5- continued

	Prey weight (g)	Coastal N prey (% Biomass*)	Inland N prey (% Biomass*)
Reptiles			
Large Lizard ^ψ	25	75 (2.71)	39 (2.97)
Medium/small Lizard ^ψ	15	114 (2.47)	49 (2.24)
Total reptiles		189 (5.17)	88 (5.21)
Insects			
Locust		22	14
Coleoptera		1	-
Mantidae		1	1
Orthoptera		2	-
Scarabidae		21	8
Unidentified		5	-
Total insects		52	23
Total prey		1197	563

*values calculated excluding insects.

^φfor coastal and inland regions, respectively (see methods)

Among reptiles, pellet analyses showed that Black Harriers mostly consumed prey of small-medium size (58.8 %), but larger prey were also common (41.1 %). We believe all of the reptiles to be lizards (i.e. Cape Skinks *Trachylepis capensis*, authors', *unpublished information*).

Diet variation

Diet composition of Black Harriers differed significantly between regions (Chi-square tests, $\chi^2 = 28.52$, d.f. = 2, $p < 0.0001$). Small mammals were more prevalent in the diet of coastal than inland nests, both numerically (67.8 % vs. 57.2 %, respectively) and in percentage of biomass (Table 5), whereas the contribution of birds was greater in inland regions, where their contribution in biomass was almost three times higher than in coastal areas (28.9 % vs. 10.8 %, respectively; Table 5). The consumption of reptiles was equivalent in both regions, both numerically (16.5 % vs. 16.3 % for coastal and inland regions, respectively) and in terms of biomass (Table 5).

We also found significant inter-annual variation in diet composition within both coastal ($\chi^2 = 28.11$, d.f. = 14, $p = 0.014$) and inland regions ($\chi^2 = 34.82$, d.f. = 6, $p < 0.0001$) (Figure 11). Considering the Coefficients of Variation, the percentage of small mammals and reptiles was more variable among years in inland (CV = 24.6 and 68.4 %, respectively) than in coastal regions (CV = 14.4 % and 50.8 %, respectively), while an opposite pattern was found for the percentage of birds, with higher variability among years in the coastal region (CV = 40.4 %) than in inland regions (CV = 25.6 %). Shannon Diversity Index in coastal regions varied among years between 0.24 and 0.44, while in inland regions it ranged between 0.35 and 0.47, being on average higher in the latter than in the former (0.41 vs. 0.33 respectively).

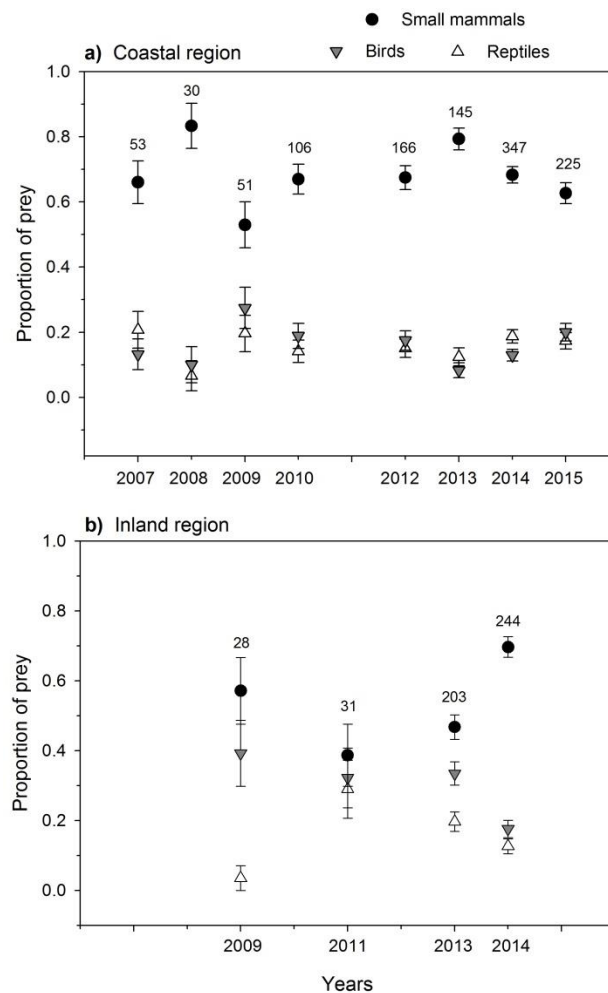


Figure 11. Inter-annual variation in the diet composition (mean occurrence \pm SE) of Black Harriers in the coastal (a) and inland regions (b). The figure only shows data for years for which we had at least 25 identified prey in a given region (see Table 4). Numbers above error bars refer to sample sizes (number of identified prey for a given year and region).

DISCUSSION

Our study highlights the importance of small mammals in the diet of breeding Black Harriers, as they represented about 65 % of the total identified prey and 78 % of consumed biomass. In this chapter, our analyses did not take into account potential variability among individuals, which potentially could have affected the results if some individuals specialise in certain prey types and if these territories are over-represented in certain years or areas. However, we do not think that this would have biased our overall results due the large data set and the widespread source of data (both spatially and throughout time). In any case, we deal with this issue more specifically in Appendix C in Chapter 3.

Overall, our results confirm that this Endangered raptor specialises in small mammals, as suggested in a previous study (Curtis et al. 2004). As with other South African raptor species (e.g. African Marsh Harrier *Circus ranivorus*, Simmons et al. 1991), our study also highlights the particular importance of the Striped Mouse in the Black Harrier's diet. This species is a common and locally abundant omnivorous rodent, described as resilient to fluctuations in environmental conditions and/or food availability, which probably explains its widespread distribution and capacity to inhabit very different biomes within South Africa (de Graaff 1981). Its high seasonal productivity as well as its diurnal habits (Perrin 1980) may also explain its importance in Black Harrier diet. Both prey and predator present similar and overlapping daily activity patterns, and therefore the Striped Mouse may be potentially more available to Black Harriers throughout its breeding season compared to other species with different activity patterns (Simmons 2000).

Species with a specialist narrow diet are frequently associated with a poorer conservation status (Ferrer & Negro 2004; Huang 2013). In these species, changes in the abundance of the primary prey lead to strong declines in reproductive success (Newton 1998; Resano-Mayor et al. 2014). Some studies have also highlighted that the degree of dietary specialisation is often related to the species' distributional range, and that specialists show smaller distribution ranges than generalists (Boyes & Perrin 2009). Specialists are also likely to be less adaptable and more vulnerable to rapidly changing environmental conditions compared to generalists (Devictor et al. 2008). Thus, the dietary dependence of Black Harriers on small mammals, and more particularly on the Striped Mouse, may

contribute to its scarcity and Endangered status, which may be further exacerbated in the context of global change.

The western region of South Africa has been intensively modified over the last century, with the anthropogenic conversion of the Fynbos and particularly Renosterveld vegetation losing over 90 % of its former area to agriculture or urbanization (Curtis et al. 2004). This has reduced and fragmented the breeding habitats of several species, including Black Harriers by > 90% in the most productive regions of the Overberg. Furthermore, there has been a shift in rainfall and temperature patterns in South Africa over the past 50 years, and most notably in the Cape Floral Kingdom and Succulent Karoo (Midgley et al. 2002; Simmons et al. 2004). Here, temperatures are predicted to become warmer and winter-rainfall more scarce, with longer and more frequent droughts (Midgley et al. 2002; Hockey et al. 2011; Cunningham et al. 2015). Given that several studies have described the importance of rainfall for the reproduction of several small mammal species, including the Striped Mouse (Taylor & Green 1976; Jackson & Bernard 2006), these two phenomena combined could lead to a reduction in the abundance and/or availability of the primary prey of specialist predators like the Black Harrier. This would force them to switch to alternative prey that are harder to catch (i.e. more energy spent chasing them) such as birds or reptiles (Temeles 1985). This in turn may lead to decreased foraging success and breeding outputs, as evident in other predatory species (e.g. Simmons et al. 1986; Korpimäki 1992; Arroyo & García 2006; Terraube et al. 2012).

However, some specialist species may show behavioural flexibility. For example, Verreaux's Eagles *Aquila verreauxii* are considered to be highly specialised on hyrax species in some areas (Boshoff et al. 1991; Davies 1994; Davies & Allan 1997), but a recent study showed that individuals breeding in agricultural landscapes had the capacity to adapt positively to their changing environment (via the incorporation of energetically profitable and highly available prey to a broader diet) without negative effects in breeding performance (Murgatroyd et al. 2016a). Within and between harrier species, there is a very wide range of diet patterns. Some species (essentially from the European continent) are generalist with broad diets, but may show specialist diets in certain contexts, like the Montagu's Harrier *Circus pygargus* (Schipper 1973; Terraube & Arroyo 2011; Limiñana et al.,

2012), the Hen Harrier *Circus cyaneus* (Schipper 1973; Redpath et al. 2001, 2002a; Millon et al. 2002; García & Arroyo 2005), and the European Marsh Harrier *Circus aeruginosus* (Schipper 1973; González-López 1991). On the other hand, some species appear to be specialised on small mammals, for example the Pallid Harrier *Circus macrourus* (Terraube et al. 2010), the Northern Harrier *Circus cyaneus hudsonius* (Hamerstrom 1986; Barnard et al. 1987), or the Black Harrier (as shown in the present study), but the degree of diet flexibility in the absence of small mammals varies among species.

It seems therefore, essential to carefully assess the factors that define dietary strategies and affect diet variation (e.g. prey abundance, availability and profitability) in this species, as well as their ecological consequences, particularly in the context of rapid and long-term environmental change. Further studies will also have to assess the long-term broader implications that diet specialisation coupled with climate change and habitat fragmentation may play on the population dynamic of this Endangered specialist predator.

Chapter 3

Regional and temporal variations in diet and provisioning rates suggests weather limits prey availability for an Endangered avian predator



While awaiting for the examination of this thesis, a modified form of this chapter has been published in the Ibis journal: *García-Hera, M.-S., Mougeot F., Simmons R.E. & Arroyo B. 2017b. Regional and temporal variations in diet and provisioning rates suggests weather limits prey availability for an Endangered avian predator. Ibis, 159: 567-579, Online, doi:10.1111/ibi.12478.*

ABSTRACT

Understanding variations in food requirements of wild animals is of central importance in population ecology and conservation, as this helps identify where and when food may be limiting. Studies on diet variation or prey provisioning rates may give useful insights when direct information on prey availability is lacking. We report here on spatial-temporal variations in the diet of an Endangered southern African predator, the endemic Black Harrier *Circus maurus*. This raptor specializes on small mammals, but also feeds on birds and reptiles as alternative prey. Using ca. 1,000 pellets collected in inland and coastal regions during 2006-2015, we show that diet composition hardly changes throughout the breeding season in the coastal region. By contrast, we show a marked seasonal decline in the occurrence of small mammal prey in the inland region, with a concomitant increase of alternative prey. Proportion of small mammals in the diet declined as maximum temperatures rose, and peaked inland late in the breeding season. Using camera recordings at nests, we further analysed daily patterns of prey provisioning to nestlings. We show that a marked reduction in small mammal prey provisioning during midday occurred in the warmer inland region, but not in the cooler coastal region. Reduced availability of the primary prey, i.e. small mammals, in hotter temperatures, through a reduction of activity or overall abundance, could explain these patterns. Finally, we also show a positive relationship between winter rainfall and inter-annual differences in the proportion of small mammals in the diet of harriers breeding in the coastal region, suggesting relationships between diet and prey abundance are mediated through weather. We discuss the need to consider spatial variations in food availability for conservation strategies.

INTRODUCTION

Food supply is a main natural limiting factor affecting birds, influencing all aspects of their annual cycle (Newton 1998), and is mainly related to food availability, determined by both the abundance and accessibility of food resources (Preston 1990). In the case of predators, factors such as habitat or weather may reduce accessibility through modifying prey behaviour or capture probability (Elkins 1983; Schlaich et al. 2015). This may create situations of food limitation despite high prey abundance (Robinson et al. 2016).

However, quantifying and measuring the availability of prey in the environment remains a difficult and challenging task for many species (Rosenberg & Cooper 1990; Smith & Rotenberry 1990). Studies on diet variation may nevertheless give relevant and useful insights on these factors when direct information on prey availability is lacking (e.g. Amar et al. 2003; Terraube et al. 2011; Cartwright et al. 2014), particularly for specialist predators. Unlike generalists, specialist predators use a narrow range of resources, feeding primarily on one food resource when accessible (see Pyke et al. 1977; Stephens et al. 2007). Changes in diet composition (i.e. a higher proportion of alternative prey in the diet) in these species arise primarily if the availability of the primary prey decreases (Newton 1998; Arroyo & García 2006). Additionally, in the absence of prey abundance data, an indirect way to evaluate food availability in the environment may be to assess foraging yields (i.e. number of individual caught per unit time of hunting, e.g. Simmons 2000; Terraube et al. 2011) or prey provisioning rates at nests via direct observations (Amar et al. 2003; Leckie et al. 2008) or using automated camera recording at nests (Zárybnická et al. 2012; Schroeder et al. 2013; Robinson et al. 2016).

For specialist species of conservation concern, it is paramount to identify under which conditions food is limiting. Specialist species are indeed known to be less efficient while hunting alternative prey (Terraube et al. 2011). In this context, a reduction in the availability of primary prey in the environment may contribute to reducing individual's breeding performance, and potentially have broader implications for the entire population (Newton 1979, 1998).

The ground-nesting Black Harrier *Circus maurus* is a scarce raptor endemic to southern Africa. Its population size has been estimated at less than 1,000 breeding individuals and the species is currently considered Endangered in South Africa, Namibia and Lesotho, which corresponds to its whole distribution range (Simmons et al. 2015; Taylor 2015). This medium-sized bird breeds in indigenous vegetation in both coastal and inland areas of south western South Africa, essentially within the Fynbos and the Karoo Biomes (Curtis et al. 2004; Curtis 2005; Chapter 1). The species has recently been confirmed as a small mammal specialist, with the Four-Striped Mouse *Rhabdomys pumilio*; hereafter "Striped Mouse") being the main prey, although it also consumes alternative prey such as birds or reptiles (Chapter 2; see also Van der Merwe 1981; Steyn 1982). Regional differences have also been found in its diet, with a greater consumption of birds inland than along the coast (Chapter 2), but the potential reasons for such differences have yet to be explored.

No quantitative data exist on the availability of the range of prey taken by this species over the landscape it occupies. However, rainfall is considered as a determinant factor of primary productivity in the most arid regions of South Africa (Schulze 1997; Lepage & Lloyd 2004; Rymer et al. 2013), and several studies have found that winter rainfall promotes the reproduction of small mammals, including the Striped Mouse (Taylor & Green 1976; Jackson & Bernard 2006). In this context, higher winter rainfall would be expected to be associated with a greater abundance of small mammals. On the other hand, high temperatures are also known to induce a reduction of the activity of South African small mammals, such as the Striped Mouse (Nater et al. 2016). Therefore, high temperatures, e.g. those occurring at mid-day as the breeding season extends into summer, would be expected to be associated with a lower accessibility of small mammals.

In this paper, our aim is to investigate the spatial and temporal variations of diet of breeding Black Harriers in South Africa. Using a large data set of 1,679 identified prey from ca. 1,000 pellets collected at active nests during 2006-2015, we looked at seasonal and regional variations of diet, and investigated diet variation according to winter rainfall and maximum temperatures. We hypothesized that the occurrence of small mammals in Black Harrier's diet should increase with winter rainfall, and decrease with increasing

temperatures. We also assessed regional differences in provisioning rates as an indirect way of evaluating differences in prey availability.

MATERIALS AND METHODS

Study sites

Fieldwork was conducted in South Africa, along the coast of the Western Cape Province (33.700° S, 18.45° E; 33.133° S, 18.083° E) and inland in the Northern Cape Province in the Nieuwoudtville area (31.316° S, 19.083° E). Nests were located in and around National Parks (South African National Parks –SANParks properties), Provincial Protected Reserves (Cape Nature), or on private lands. We classified each nest either as coastal or inland, depending on their location and altitude: coastal nests were defined as those within 15 km from the coast and with a maximal altitude of 100 mASL, and those located further than 15 km from the coast and with an altitude higher than 100 mASL were considered as inland (see Chapter 1 for more details). Inland Black Harrier nests were mostly within the Karoo biome, whereas those along the coast were mostly within the Fynbos biome (see Mucina & Rutherford 2006; Manning 2007; Chapter 1 for details on vegetation types).

Pellet collection and prey identification

Black Harrier diet was assessed through the analyses of pellets collected at active nests and nearby perching sites, such as posts or dead bushes known to be used by a specific breeding pair. Pellet contents (i.e. remains such as bones, scales, feathers or hairs) were analysed following the methods described in Chapter 2. Briefly, prey were identified to the lowest possible taxon level (i.e. to species level when possible, or broader categories such as “small mammals”, “passerines”, “galliformes” or “reptiles” if not), and the minimum number of individuals per taxon for each pellet was determined. This was based on the highest number of mandibles, incisors, skulls, bills or limbs, where they occurred, and assuming only one individual when only fur, feathers or scales were found. However, for a sub-sample of pellets with fur only (n= 105), hair imprint analysis allowed us to identify if more than one small mammal was present (Chapter 2 for details).

We further estimated the relative biomass for each prey as follows: 1) for prey identified to species levels, using the average weight described for the species in Stuart & Stuart (2015); 2) for broader prey categories (e.g. “unidentified small mammals” or “unidentified birds”), using an estimated average weight, based on that of identified prey of the same taxonomic level (details in Chapter 2).

Pellet collection occurred between July-December in the coastal region and September-December in inland region (Chapter 2), covering both the incubation and the nestling periods of Black Harriers in both areas. Each pellet was attributed to a region (coastal or inland) and a “month of collection” (i.e. the month as when the pellet was collected in the field). For simplicity, we henceforth refer to months of collection as “month”.

A total of 953 pellets was collected during the 2006-2015 breeding seasons from the two study regions ($n = 659$ and 294 for coastal and inland, respectively) at 111 active nests (79 coastal and 32 inland). We excluded from analysis 2 pellets found in July (one in 2006 and one in 2011, totalling 6 identified prey) because they could not be confirmed to correspond to breeding birds. In the coastal region, pellets were collected in all study years, while in the inland region they were collected only in 2006, 2008, 2009, 2011, 2013 and 2014. Overall, the total number of identified prey was 1685, and we analysed 1679 after excluding those found in July. For analyses, we classified all identified prey into one of three categories: small mammals, birds or reptiles.

Provisioning rates

To determine provisioning rates, we set cameras (Ltl Acorn-6210M, 32GB SD card) at 18 active nests (11 coastal, 7 inland) during the 2014 breeding season. Cameras were set 100-150 cm from the edge of the nest during the nestling period, when chicks were 7 to 41 days old, and were camouflaged and covered with vegetation to avoid disturbing the breeding harriers and attracting potential nest predators. On average, each nest was monitored for 10 days throughout the nestling period, but not necessarily continuously (due to SD memory card or camera battery limitations). In the coastal region, 10 nests were monitored between 28 August and 27 October, except for one pair that started breeding later and for which

recordings were made from 12 November to 3 December. Inland, all 7 nests were monitored between 6 - 31 October. Cameras were either set to shoot an image every 5 seconds, or to record a 60 second video sequence (with a time lapse of 1 second between two consecutive videos), from sunrise until sunset (e.g. 06h00 – 19h59). We obtained a total of 1,557 videos and 90,692 minutes of recordings (527 ± 40.2 min per nest, range 397–665 min). We analysed videos to identify when prey was delivered, and categorized each prey as a small mammal, bird, reptile or unidentified prey item. We used these data to compare average provisioning rates and their diurnal patterns between the study regions. We could not use these data to assess seasonal declines in provisioning rates, because cameras were not active for long enough. For those nests for which we had both pellets and cameras, diet identified with both methods correlated with each other when the proportion of unidentified prey in cameras was low (see Appendix B). When the proportion of unidentified prey was high, the camera data became less reliable. We preferred not to lump prey identified in cameras with those in pellets for analyses of diet variation for the one year (2014) when both methods were used, and to use only pellet data, as this rendered more comparable data across study years.

Rainfall and maximum temperature

Winter rainfall (June-September) is known to affect the onset of small mammal reproduction as well as their subsequent abundance (Rymer et al. 2013; Nater et al. 2016). Monthly rainfall data (mm) from 7 weather stations located near study nests in the Western and Northern Cape Provinces were obtained for the periods 2006-2015 (South African Weather Services 2015). We calculated total rainfall between June and September for each weather station and each year. We linked diet data from a given breeding site to the winter rainfall data calculated from the nearest weather station.

Additionally, high temperatures during the middle of the day are known to reduce activity of small mammals (Nater et al. 2016), thus reducing their availability as prey for harriers. Monthly maximum temperature data ($^{\circ}\text{C}$) from the 7 weather stations for each of the study years were also obtained, and linked to diet data from a given breeding site and collection month (nearest weather station). The distance between sampled nests and weather stations averaged 29 ± 22 (SD) km.

Statistical analyses

We used R 3.2.3 (the R Foundation for statistical computing, 2015) for all statistical analyses.

Regional and seasonal variations in diet

We wanted to test the effects of month (seasonal variations), region, winter rainfall and maximum temperature on the occurrence of the different prey groups (small mammals, birds and reptiles) in Black Harrier diet. However, some of the above-mentioned variables are inter-linked, and could thus be collinear. So, we first investigated whether winter rainfall differed between years and regions, and whether maximum temperatures differed between years, regions and months. For this, we conducted Generalised Linear Mixed Models (GLMMs), where each response variable (winter rainfall and maximum temperature) was fitted as a normal distribution (package lme4, function lmer and log function; Bates et al. 2012). In the model for winter rainfall, year (10-level factors) and region (2 level factor: coastal vs. inland) were fitted as explanatory variables, while in the models for maximum temperature, year, region, month (continuous variable ranging from 8 August to 12 December), and the interaction region x month were fitted as explanatory variables. In both models, weather station was included as a random term to account for the non-independence of weather data from the same station. These analyses showed that maximum temperature was collinear with both region and month (see results).

We analysed the influence of these factors on variation in the occurrence of the different prey groups in the diet using GLMMs. For this, our sample unit was each identified prey. We constructed three response variables (occurrence of small mammals, birds or reptiles) by attributing 1 to prey corresponding to a particular group, and 0 when it corresponded to any other prey group (e.g. occurrence of small mammals would be scored 1 for all small mammal prey, then 0 for both birds and reptiles). Response variables were fitted using a binomial distribution (package lme4, function glmer and a logit link function; Bates et al. 2012). We included year (10-level factor) and pellet ID as random terms: the former to account for potential between-year variation in diet for reasons not taken into account in our analyses (and because we were interested in describing overall patterns for any given year, rather than testing for between-year variation here); and the latter (pellet ID) to take into account potential lack of independence for data arising from the same

pellet. However, as maximum temperature was collinear with both region and month (see results), we could not include those three variables in the same model. Thus, we first fitted a model with month, region, their interaction and winter rainfall as explanatory variables, and a separate one with maximum temperature instead of month and region. This was to check whether differences in temperature could explain observed seasonal and regional patterns.

While “month” corresponded to the month when pellets were collected, an exception was made for pellets collected at inland nests in 2014. That year, only two visits to the inland study site were conducted: at the beginning and end of October. For this year, and in order to incorporate temporal variability, we attributed pellets collected in the first visit ($n = 62$) to September as the month of collection. All nests found during the first visit (i.e. early October) were either still at the incubation stage, or had young nestlings of approximately 2-5 days old, and we could then reasonably assume that most pellets collected then were indicative of the adult diet during the month prior to collection. Additionally, 21 pellets collected in the first days of January 2008, 2009 and 2013, were attributed to the previous December as the month of collection, given that birds were no longer present then and we assumed that they represented diet during the previous month.

A stepwise backward procedure was performed for model selection (with the function `drop1`), where likelihood ratio χ^2 tests (LRT) on the residual sum of squares of models with and without interactions or individual variables were used to select the best models (Zuur et al. 2009).

Inter-annual variations in diet with weather

To investigate whether diet composition was associated with winter rainfall, we used Pearson correlations between winter rainfall and the overall proportion of each prey type in the diet for each study year. These analyses were conducted using only data from the coastal region, where potential sampling bias (arising from the month of collection) across years was not an issue because no seasonal variation in diet was found there (see Results). We excluded data from 2011 because only three prey were identified that year. For the inland region, we could not conduct a similar analysis because sampling in each year

occurred in different months, complicating annual estimates of diet in a region where seasonal differences were apparent.

Regional variations in daily provisioning rates

To test for differences in daily provisioning rates between regions, we fitted the number of prey delivered at each monitored nest ($n = 18$ nests) during a given hour as the response variables (total prey items, small mammals, birds, reptiles and unidentified prey items) using GLMMs (Poisson distribution, package lme4, function glmer and logit link function; Bates et al. 2012), and the log of duration of the “recording time” fitted as an offset. “Recording time” was 60 min if the camera had been active for the whole hour, but less if it had been inactive for part of that time. Hours with recording time < 30 min were excluded from analyses ($n = 78$). We included nest identity as a random term to account for the lack of independence of data coming for the same nest. We categorized recording hours (06h00-19h59) in three categories (hereafter referred as “daytime periods”) as follows: morning (06h00-10h59), midday (11h00-15h59) and evening (16h00-19h59). Daytime periods (3-level factor), region and their interaction were included as explanatory variables.

As above, a stepwise backward procedure was performed for model selection (with the function drop1), and likelihood ratio χ^2 tests (LRT) on the residual sum of squares of models with and without interactions or variables were used to select the best models (Zuur et al. 2009). All data are presented as means \pm SE.

RESULTS

Temporal and spatial variations in weather

Winter rainfall varied significantly among years ($\chi^2 = 56.5$, d.f. = 9, $p < 0.0001$) but did not differ between regions ($\chi^2 = 0.15$, d.f. = 1, $p = 0.69$). Maximum temperature increased significantly with month ($\chi^2 = 174.5$, d.f. = 1, $p < 0.0001$; 1.85 ± 0.15), varied significantly among years ($\chi^2 = 31.5$, d.f. = 9, $p < 0.0002$) and differed between regions ($\chi^2 = 8.2$, d.f. = 1, $p = 0.004$; LS means \pm SE: 23.0 ± 0.94 and 26.7 ± 0.90 for coastal and inland regions, respectively). The interaction month \times region was not significant ($\chi^2 = 0.01$, d.f. = 1, $p = 0.91$).

These results indicated that maximum temperature co-varied with region and month and these variables were therefore, collinear. Of all years in the inland region, 2008 was the hottest and wettest, and 2011 was the coolest and driest (Table 6).

Table 6. Average maximum temperature and rainfall per year (2006-2015) and for each region (coastal and inland).

	Year	Average maximum temperature August-December \pm SD	Average rainfall June-September \pm SD
Coastal region	2006	16.8 \pm 0.3	81.6 \pm 9.5
	2007	23.6 \pm 4.0	176.9 \pm 48.4
	2008	21.6 \pm 1.3	244.6 \pm 0
	2009	22.0 \pm 2.1	111.4 \pm 55.1
	2010	21.9 \pm 1.0	89.6 \pm 45.6
	2011	21.1 \pm 0.6	100.9 \pm 53.0
	2012	23.9 \pm 2.4	208.5 \pm 37.2
	2013	20.5 \pm 2.5	271.6 \pm 4.3
	2014	24.7 \pm 1.8	178.6 \pm 2.3E-05
	2015	24.7 \pm 1.4	102.8 \pm 9.8E-06
Inland region	2006	27.1 \pm 2.4	169.2 \pm 86.5
	2008	29.4 \pm 1.0 E-6	280.2 \pm 8.8E-06
	2009	26.9 \pm 0.4	280.2 \pm 7.8E-6
	2011	26.8 \pm 1.5	91.1 \pm 49.7
	2013	26.8 \pm 2.1	231.1 \pm 69.9
	2014	28.2 \pm 2.3E-6	127.2 \pm 66.8

Regional and seasonal variation in diet

Dietary variation throughout the breeding season differed between regions. Inland, a clear decline in the occurrence of small mammals was observed as the breeding season progressed (September-December; Figure 12b); they were mostly replaced by birds and some reptiles (i.e. lizards). Indeed, the relative proportion of bird numbers and biomass in the diet doubled between September and December, when it reached 40 % of the total biomass provisioned (Figure 12b, d). By contrast, at coastal nests, diet composition was

stable throughout the breeding months (August-December), with a clear predominance of small mammals representing 70 – 85 % of the monthly biomass intake (Figure 12a, c).

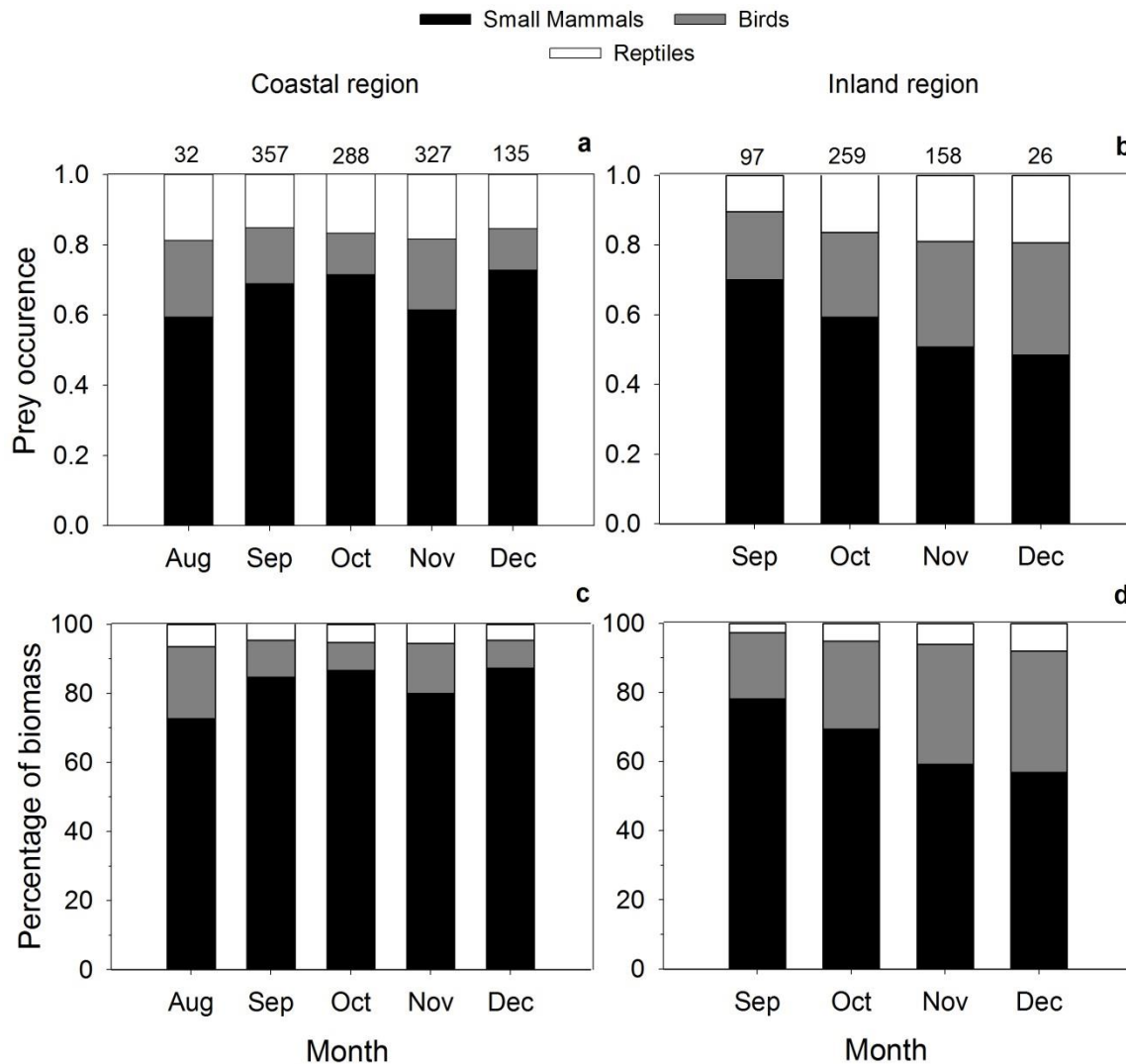


Figure 12. Monthly variation in the proportion of small mammals (black bars), birds (grey dark bars) and reptiles (white bars) in the diet of breeding Black Harriers. (a) and (c) respectively show the occurrence and the percentage of biomass in the coastal region, whereas (b) and (d) respectively show the occurrence and the percentage of biomass in the inland region. For coastal regions, proportion of prey was calculated for 2006-2010 and 2012-2015, while for inland regions this was only available for the years 2006, 2008, 2009, 2011, 2013, and 2014. Sample sizes (number of identified prey) are indicated above bars, and excluded the prey identified in July (n= 1,679 total identified prey).

These differences in diet were further confirmed by GLMMs models, which showed a significant region x month interaction explaining the occurrence of small mammals in the diet (Table 7 and Figure 13). The interaction reflected a strong negative relationship between the occurrence of small mammals and month in the inland region (slope: -0.40 ± 0.14) that was almost non-existent along the coast (slope: -0.006 ± 0.06) (Figure 13). When removing the interaction region x month from the model, small mammal occurrence in diet differed significantly between regions ($\chi^2 = 17.31$, d.f.= 1, $p < 0.0001$), and was significantly greater in the coastal (LS means: 0.68 ± 0.09) than in the inland region (LS means: 0.19 ± 0.13). These differences in diet composition, found at the population level, were also found at the nest level (see Appendix C). No effect of winter rainfall was found on the within-year occurrence of small mammals after taking regional and seasonal factors into account ($\chi^2 = 0.03$, d.f.= 1, $p = 0.87$).

The occurrence of birds in the diet also varied significantly between regions (Table 7), being greater inland (LS means: -0.81 ± 0.16) than along the coast (LS means: -1.60 ± 0.13), but did not vary with month ($\chi^2 = 0.002$, d.f.= 1, $p = 0.96$). Additionally, after taking regional variation into account, the occurrence of birds in the diet tended to increase with winter rainfall (Table 7, slope: 0.13 ± 0.07).

Seasonal variation in the occurrence of reptiles in the diet also tended to differ between study regions (marginally significant interaction of region x month; Table 7), with a seasonal increase in the inland region (slope: 0.33 ± 0.17) and no apparent trend in the coastal region (slope: 0.03 ± 0.07). When removing the interaction region x month from the model, the occurrence of reptiles was not significantly different between regions ($\chi^2 = 0.02$, d.f.= 1, $p = 0.90$). Winter rainfall did not explain reptile occurrence in the Black Harrier's diet ($\chi^2 = 0.41$, d.f.= 1, $p = 0.52$).

When considering maximum temperature instead of region and month, we found that the occurrence of small mammals significantly declined with increasing temperatures ($\chi^2 = 11.31$, d.f.= 1, $p = 0.0007$; slope: -0.18 ± 0.05), suggesting that the seasonal regional differences in diet could be linked with differences in maximum temperature. Accordingly, the occurrence of birds and reptiles increased with maximum temperature ($\chi^2 = 5.9$, d.f.= 1, $p = 0.01$, slope: 0.16 ± 0.06 and $\chi^2 = 3.19$, d.f.= 1, $p = 0.07$, slope: 0.12 ± 0.06 , respectively).

Table 7. Results of the Generalized Linear Mixed Models (GLMMs) testing for variation in the occurrence of small mammals, birds and reptiles in the Black Harrier's diet. Initial models included region (coastal or inland), month (August-December), and their interaction as explanatory variables. The table shows the best models (the results of comparing models with and without those variables) after stepwise backward selection. When the best model included a significant interaction, individual effects were also maintained. Year (2006-2015) and pellet ID were included as a random effect in all models. d.f.: degree of freedom.

Dependent variables	Explanatory variables	d.f.	Chi-square	P
Small mammal occurrence				
	Month x Region	1	7.69	0.006
Bird occurrence				
	Region	1	28.83	< 0.0001
	Rainfall	1	3.12	0.08
Reptile occurrence				
	Month x Region	1	3.50	0.06

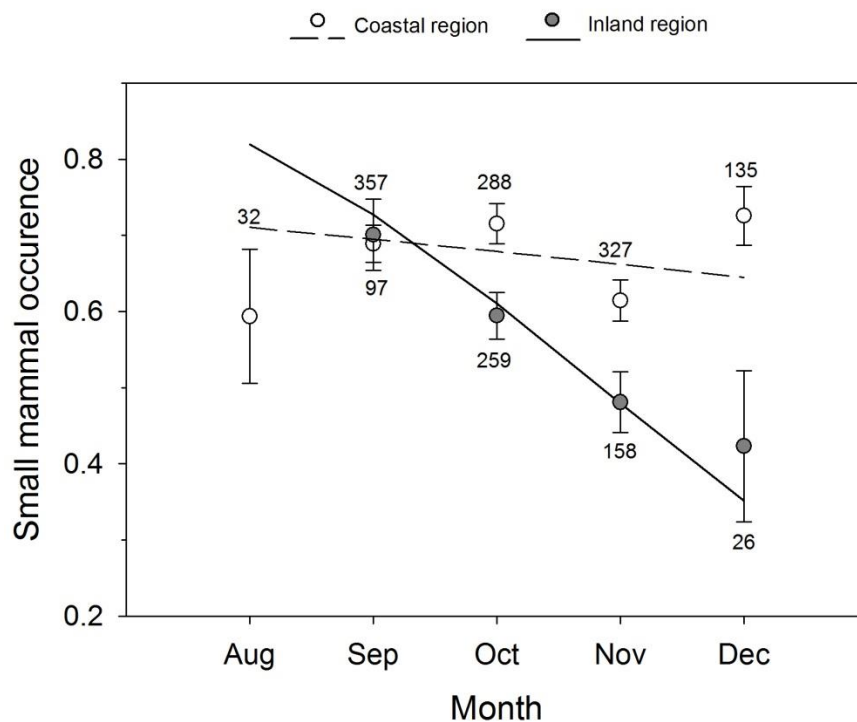


Figure 13. Monthly variation in the occurrence of small mammals in the diet of Black Harriers breeding in coastal (white circles/dashed line) and in inland (grey dark circles/ solid line) regions. Lines represent modelled data from the GLMM results. Raw data (circles) are also shown. Sample sizes (number of identified prey) are indicated above/below the error bars.

Inter-annual variations in diet with weather

In the coastal region, inter-annual variations in diet appeared to be correlated with winter rainfall. The average proportion of small mammals in the diet increased with the amount of winter rainfall (Pearson correlation, $r = 0.83$, $p = 0.005$) varying from 46 % for years with little rain, up to 83 % for wetter years. Conversely, the proportion of birds and reptiles decreased with increasing winter rainfall ($r = -0.85$, $p = 0.004$ and $r = -0.65$, $p = 0.06$, respectively; Figure 14). Bird occurrence varied from 31 % during years with little rain, down to 1 % during wetter years, and reptile occurrence varied from 23 % for years with little rain, to < 1 % for wetter years. The annual proportion of each prey type was not significantly related to the yearly average maximum temperature during the breeding months (all $p > 0.2$).

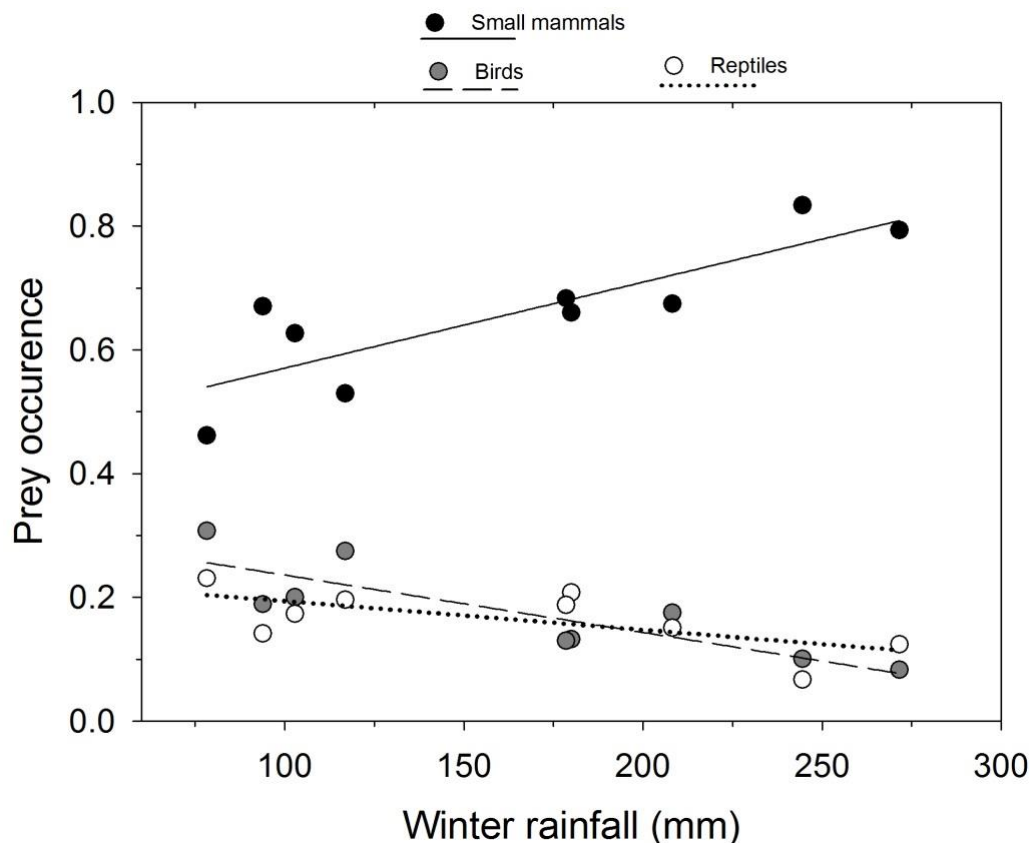


Figure 14. Relationships between winter rainfall [sum of rainfall June-September (mm)] and the yearly occurrence of small mammals (black circles / solid line), birds (dark grey circles / dashed line) and reptiles (white circles / dotted line) in the diet of Black Harriers breeding in the coastal region. Data from the years 2006-2010 and 2012-2015 were analysed.

Provisioning rates

Variation in overall provisioning rates, i.e. total items delivered to the nest per hour, was explained by a significant interaction between regions and daytime period (Table 8), indicating that diurnal patterns of provisioning differed significantly between regions. At coastal nests, provisioning rate was similar during mornings (0.81 ± 0.04 prey/hr) and midday (0.85 ± 0.04), and decreased slightly during evenings (0.65 ± 0.04). At inland nests, by contrast, a marked drop in provisioning rates occurred during midday (0.42 ± 0.03) compared with mornings (0.59 ± 0.04) and evenings (0.64 ± 0.06) (Figure 15a). On removing the interaction region x daytime period, the provisioning rates significantly differed between regions ($\chi^2 = 13.73$, d.f. = 1, $p = 0.0002$) and were on average 46 % greater in the coastal (0.78 ± 0.02 prey/hr) than in the inland region (0.53 ± 0.02 prey/hr).

Table 8. Results of the Generalized Linear Mixed Models (GLMMs) testing for differences between daytime periods and regions in prey provisioning rates (prov. rate) to nestlings (all prey items, small mammals, birds, reptiles and unidentified items). Initial models included region (coastal vs. inland), daytime period (morning, midday, evening) and their interaction. The table shows the best models (the results of comparing models with and without those variables) after stepwise backward selection. When the best model included a significant interaction, individual effects were also maintained. Nest identity was included as a random effect in all models. d.f.: degree of freedom.

Dependent Variables	Explanatory Variables	d.f.	Chi-square	P
All prey item prov. rate	Region x Daytime period	2	11.74	0.003
Small mammal prov. rate	Region x Daytime period	2	12.67	0.002
Bird prov. rate	Region x Daytime period	2	5.66	0.06
Reptile prov. rate	Daytime period	2	7.99	0.02
Unidentified-prey prov. rate	Region	1	5.22	0.02

Similar patterns were found when we examined provisioning rates of small mammal prey, for which a significant interaction between regions and daytime period was also found (Table 8). At coastal nests, small mammals were delivered at a similar rate during mornings (0.48 ± 0.03) and midday (0.55 ± 0.03), but at a lower rate during evenings (0.38 ± 0.02). At

inland nests, a marked drop in small mammal provisioning rate was observed at midday (0.21 ± 0.02), relative to morning (0.33 ± 0.02), and evenings (0.37 ± 0.03); Figure 15b). Overall, the small mammal provisioning rate was 63 % greater in the coastal than in the inland region (0.49 ± 0.01 and 0.30 ± 0.01 , respectively).

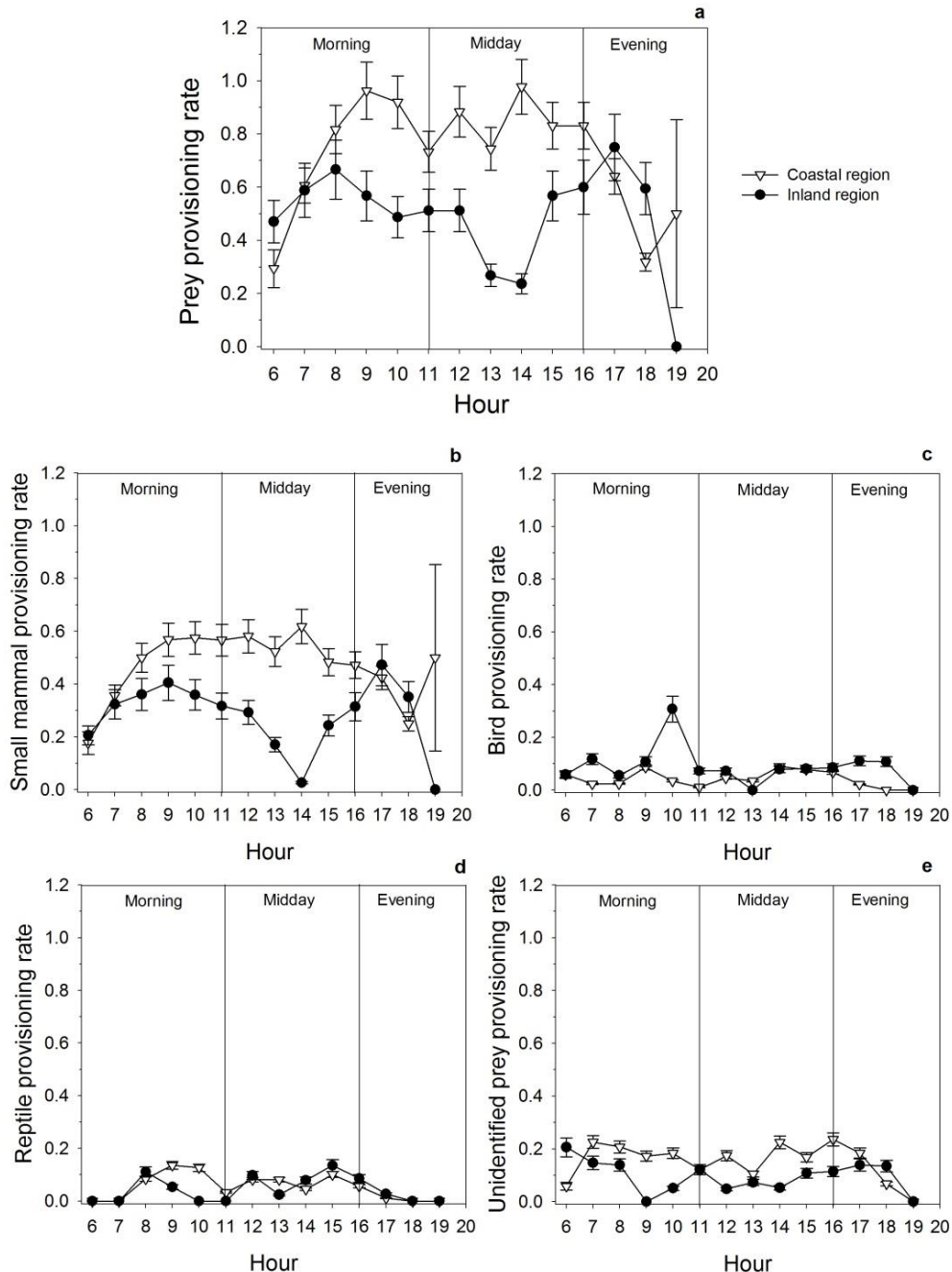


Figure 15. Daytime variation in average (\pm SE) provisioning rates (prey deliveries per hour) at Black Harrier nests ($n = 18$) according to region (coastal in white triangles; inland in black dots). Provisioning rates/hr of all prey (a), small mammals (b), birds (c), reptiles (d) and non-identified prey (e). Vertical lines separate the three analysed daytime periods (morning, midday and evening).

Provisioning rates of birds also varied between regions and diurnally (Table 8). Birds were delivered at a lower rate during evenings (0.030 ± 0.002) than during mornings (0.043 ± 0.002) or midday (0.052 ± 0.002) in the coastal region, whereas in the inland region birds were delivered at a lower rate during midday (0.061 ± 0.004), but at a similar rate during mornings (0.13 ± 0.01) and evenings (0.10 ± 0.01) (Figure 15c).

Provisioning rates of reptiles only varied diurnally, without regional differences (Table 8): reptiles were delivered about three time more often during mornings (0.070 ± 0.003), or midday (0.067 ± 0.003), than evenings (0.026 ± 0.001) (Figure 15d).

Provisioning rates of non-identified items only varied among regions (Table 8), being more frequent along the coast (0.170 ± 0.005) than inland (0.100 ± 0.005), whereas no diurnal patterns were found (Figure 15e).

DISCUSSION

Diet composition of Black Harriers differed between the two study regions, with a greater proportion (and contributed biomass) of small mammals apparent in coastal regions than inland, where birds and reptiles were relatively more frequent. Interestingly, overall differences between regions arose through seasonal changes in diet: early in the season (September), diet was similar in both regions, but inland it gradually shifted to fewer mammals with an increasing representation of birds and reptiles.

A first possible explanation for this seasonal shift in diet could be that inland breeders have more opportunities of hunting birds later in the season. Inland nests were often surrounded by a mosaic of natural vegetation, pastures and occasionally wheat fields, which may increase the availability of alternative food resources such as reptiles or grassland birds like Common Quails *Coturnix coturnix* (Ogada 2009; Murgatroyd et al. 2016a). However, on inspection of provisioning rates, we found that, overall, the frequency of birds or reptiles delivered to inland nests was lower than at coastal ones, suggesting a constraint rather than an adaptive or opportunistic shift. In other words, the observed seasonal dietary shift in the inland region probably arose from a general decrease in the

availability (i.e. abundance and or/ accessibility) of the Black Harriers primary prey (small mammals) in that region later in the breeding season.

Prey provisioning rate analyses showed that provisioning rates for small mammals were reduced during the middle of the day at inland nests, whereas alternative prey such as birds and reptiles remained accessible and available throughout the day. This supports the idea that the reduced availability of the primary prey may be at least partly explained by temperature-mediated accessibility. Some studies have indeed demonstrated that the Striped Mouse shows behavioural flexibility in response to daily temperature fluctuations in the succulent Karoo biome. In this region, the temperatures at midday can exceed 40°C during summer days (i.e. the latter half of the Black Harrier breeding season), explaining why the peak of activity for the Striped Mouse at that time occurs at dusk and dawn (Perrin 1981; Haim et al. 1998; Schradin & Pillay 2004; Nater et al. 2016). The seasonal decline in the occurrence of small mammals in the Black Harrier's diet may thus arise because they become less accessible to foraging Black Harriers during the increasingly hotter midday periods. Accordingly, our models using the monthly average of maximum temperatures revealed that the occurrence of small mammals in Black Harrier's diet decreased significantly with increasing temperatures. Overall, our results support the idea that hotter temperatures reduce small mammal availability (either accessibility through diurnal rhythms, abundance through reduced mice reproduction, or both). We cannot discount the additional, but not mutually exclusive possibility, that high temperatures are detrimental to the harriers themselves, causing them to reduce hunting effort at midday particularly at inland sites. This is supported by the observation that breeding birds seek shade at midday, nest on south-facing slopes and return to their nests to shade their young in full midday sun (RES, *personal observations*).

Higher temperatures may also be indicative of poorer environmental conditions and a concomitant reduction of small mammals' reproduction and their overall abundance. This is supported by the finding that the breeding season of the Striped Mouse is shorter (i.e. 3 months) in the succulent Karoo biome, the most frequently used inland habitat by breeding Black Harriers, than in the Fynbos biome. Here not only are all coastal nests located, but the Striped Mouse reproduction is twice as long (i.e. 6 months; Schradin & Pillay 2004; Rymer et

al. 2013), essentially due to a milder Mediterranean-like climate (Mucina & Rutherford 2006). It is also possible that regional differences in prey availability and diet also depend on factors other than temperature, such as habitat, which may modulate the effect of temperature on small mammal behaviour (Meynard et al. 2012), but this would need to be examined in further studies.

We found no association between winter rainfall and small mammal occurrence in the diet after accounting for seasonal and regional differences for all nests. This may arise because seasonal variation in diet of inland breeders is mostly influenced by temperature (influencing small mammal availability, as explained above) and winter rainfall did not differ between regions during the study years.

However, inter-annual comparisons for the coastal breeding sites alone revealed a positive association between the amount of winter rainfall and small mammal occurrence in the diet the same year, suggesting that, at least in this region, winter rainfall positively influences the abundance of Striped Mice. Some studies have also shown that the Striped Mouse abundance also varies with winter rainfall (June-August) in the succulent Karoo biome, where rain promotes the development of small succulent plants, which provide essential food for initiating reproduction and, in turn, increases mouse abundance (Schradin & Pillay 2004; Jackson & Bernard 2006; Rymer et al. 2013). So it is possible that rainfall also influences the overall annual proportion of mice for inland harrier nests in this habitat. While, winter rainfall probably interacts with temperature in explaining small mammal availability to inland harriers, our limited number of sample years for inland nests disallowed us investigating further inter-annual variation in diet there and its relation to both rainfall and temperature. But this should be contemplated in future studies.

We also found that winter rainfall tended to be positively associated with the occurrence of birds in the diet. This suggests that, as alternative prey, birds are preferentially taken (in relation to reptiles) when winter rainfall has been greater, and therefore that winter rainfall may also have a positive influence on local bird abundance. This is corroborated by studies that show that the presence of the frequently taken Common Quail *Coturnix coturnix* in the succulent Karoo biome is greater following high winter rainfall (Taylor 2005; RES and F. Van der Merwe *personal observations*). Thus, our

results overall suggest that winter rainfall positively influences the abundance of both small mammals and birds in both regions, but that small mammals will be preferred by Black Harriers when available, and that their availability is also influenced by temperature, which further shapes diet composition.

Among specialist predators, when the availability of the primary prey species declines in the environment this is generally mirrored by a decline in reproductive success (Ferrer & Negro 2004; Resano-Mayor et al. 2014). Seasonal declines in breeding performance could be thus also be associated with a deterioration of food conditions in the environment, such as certain prey types becoming less abundant or less accessible later in the breeding season (e.g. Bechard 1982; Widen 1994; Ontiveros et al. 2004). In the Black Harrier, an overall seasonal decline of all breeding parameters was found, but these declines were more pronounced in the inland than in the coastal region for clutch size and productivity (Chapter1). We suggest therefore, that the steeper seasonal decline observed inland is most likely associated with an overall decline in the availability of small mammals throughout the season there. A similar pattern has for example been found in Mauritius Kestrels (*Falco punctuates*) (Cartwright et al. 2014). In this species, the seasonal decline in breeding success was stronger for those territories that contained a higher proportion of agricultural land, and this was partly explained by a lower availability of native Geckos, the preferred prey, late in the season in agricultural land (Cartwright et al. 2014).

In the case of Black Harriers, however, neither clutch size nor productivity were, on average, significantly different between regions, indicating that differences between regions early and late in the season balanced each other out (Chapter 1). Future studies in Black Harriers should investigate the link between diet, prey availability and breeding performance at the individual level, and how this varies in space and time, in order to explore this hypothesis.

CONCLUSIONS AND CONSERVATION IMPLICATIONS

This study provides evidence of a spatial and temporal (diurnal, seasonal and inter-annual) variation in dietary composition in an African specialist raptor, which is probably caused by

variations in the availability of the primary prey (small mammals), mediated through rainfall and temperature. The seasonal decline in the occurrence of small mammals in the diet of inland harriers coincides with a steeper decline in breeding performance there (Chapter 1), suggesting that the lower prey availability in the inland region later in the season is probably limiting reproduction there. This concurs with the observation that breeding events were spread over a shorter period in the inland region (Chapter 1). Conditions for successful breeding, in terms of food availability, mediated through weather and climate, may therefore, frequently not be met outside the coastal region. This may provide a limit for the breeding range, which in turn could explain the scarcity of this species. Furthermore, differences in breeding suitability between regions may be exacerbated with climate change, as western regions of the country are expected to become warmer and rainfall more scarce, with longer and more frequent droughts (Simmons et al. 2004; Hockey et al. 2011; Cunningham et al. 2015). This may further limit the breeding suitability of the inland region for Black Harriers, if prey availability is limited both by rainfall (inter-annually) and by temperature (diurnal accessibility). Additionally, this part of South Africa, particularly the Renosterveld with richer soils on the southern coast, has been intensively modified and fragmented by agriculture over the last century (Curtis et al. 2004). This has reduced the breeding and hunting habitats of several species, including the Black Harrier. Given our results, it would be particularly important to reverse such fragmentation in coastal regions where environmental conditions are more favourable for breeding Black Harriers. Conservation measures have already prioritized the protection of Fynbos vegetation, through the creation of national parks and private reserves. However, a strengthening of the law, reinforcing the protection and conservation of these natural habitats, with the strict prohibition of further habitat destruction, would help to avoid further fragmentation of natural habitats. This would then ensure that coastal regions act as source areas for the sustainability of the global Black Harrier population, and ultimately ensure the sustainable conservation of the species.

Appendix B. Assessing diet composition using two methods: pellet analyses and image footage analyses

Materials and Methods

In 2014, cameras (Ltl Acorn-6210M, 32GB SD card) were set at 18 active nests during the nestling period, 11 from the coastal region and 7 from the inland region. Pellets from those nests were also collected, even though their number strongly varied among monitored nests (average \pm SE: 19.3 ± 0.25 , min-max: 1-69). The low numbers of collected pellets in certain nests ($n = 5$ nests, average collected pellets \pm SE: 5.6 ± 0.74 , min-max: 1-9) were either due to difficulties in finding posts that adult Black Harrier used to perch and regurgitate the pellets or because of nest failure early in the breeding season. Here, we assess whether diet composition estimated through pellet analyses was equivalent to that estimated using video and picture footages (hereafter image footage). Using image footage analyses, the identification of prey was not always possible for two main reasons: 1) some females faced away from the camera, positioning themselves between the camera and the nestlings so that the prey brought in was mostly hidden by their bodies; 2) the quality of the videos/pictures was sometimes bad due to the presence of water particles that condensed on the camera objective lens. This mostly happened at twilight, just before sunrise, due to changes in temperatures between night and day, making the footages very blurry. While prey deliveries were still detected, even in blurry footage, accurate identification of the prey was sometimes challenging. Thus, from the total number of prey delivered to nests captured on cameras ($n = 1,087$), we were able to identify a total of 837 prey (77 %), while the rest remained unidentified. For some nests, the percentage of unidentified prey was as high as 59.1 %, while for others it was quite low (i.e. 9 %). It is possible that unidentified prey is not randomly distributed among the main prey types. For analyses, we thereby decided to take into account only those nests for which we had at least 80 % of prey identified in image footages (Table B1) to get a better estimation of diet composition. From the initial 18 nests with video/picture recordings, only 9 were subsequently selected for further analyses.

Pearson correlations were conducted on the proportion of each of small mammal, bird and reptile prey in relation to all identified prey assessed with pellet analyses and with image footage. We also conducted similar analyses with the proportion of biomass attributed to each prey type (see Chapter 2 for details), as the relative contribution in terms of biomass has been shown to be important when dealing with prey categories of diverse size (Arroyo 1997).

Results and Discussion

We used R 3.2.3 (the R Foundation for statistical computing, 2015) for analyses. Pearson correlations for the proportion of small mammals as identified from pellets and image footages were both significant: $r = 0.66$, $p = 0.05$ and $r = 0.71$, $p = 0.03$, for numbers and biomass respectively (Figure B1). Similar results were found with the Pearson correlations conducted on the proportion of birds as identified from pellets and image footages: $r = 0.76$, $p = 0.016$ and $r = 0.84$, $p = 0.004$, for numbers and biomass respectively (Figure B2). These results suggest that estimations of the contribution of small mammals and birds to the diet are equally accurate with pellet analyses or image footage analyses. However, the Pearson correlation of the proportion of reptiles as identified from pellets and image footages, showed only a marginally significant relationship ($r = 0.61$, $p = 0.08$) (Figure B3a). This suggests that either pellet analyses or image footage analyses bias the actual proportion of reptiles in the Black Harriers diet. It has been well documented that prey of small size, such as lizards or insects, are often not well detected in video/picture footages when brought back into the nest by adult birds (Robinson et al. 2015). In Black Harrier pellets, the presence of reptiles (i.e. lizards) was easily determined by the presence of scales, or left and right mandibles, maxillaries, or limbs (see Chapter 2). In this context, pellet analyses could be more accurate to quantify the presence of reptiles. Furthermore, no significant relation was found between the proportion of reptile biomass assessed with pellets and that assessed with image footage ($r = 0.31$, $p = 0.41$) (Figure B3b). This suggests that one of the two methods used to estimate the biomass of reptiles may be also biased. In pellets, reptiles were divided into two size categories, large and medium-small, based on the size of the largest scales within a pellet, i.e. ≤ 2 mm for medium-small and ≥ 2 mm for large (see

Chapter 2). On the image footage the size of the prey was estimated qualitatively in relation to the tarsus length of the adult females.

Overall, however, we can conclude that the assessment of Black Harrier's diet composition, in terms of the relative proportion of prey and biomass, using pellet analyses or video/pictures from cameras set at nests, was equivalent for larger size prey. However, precaution has to be taken when looking at small size prey, such as reptiles. Because the overall contribution of reptiles to diet is small, these biases may be unimportant when using diet data to compare broad diet categories dominated by small mammals, versus more diverse diets including alternative prey.

Table B1: Black Harrier active nests monitored with cameras in the coastal and the inland region in 2014 (n= 18). For each nest, the total number of prey, and the percentage of small mammals, birds, reptiles and unidentified prey is given. Nests in bold were those with at least 80 % of all prey identified and used for further statistical analyses.

Nests	Total number of prey	% Small mammals	% Birds	% Reptiles	% Unidentified prey
Coastal region					
Rondeberg 1_14*	49	0.80	0.02	0.02	0.16
Silverstroom 1_14	93	0.41	0	0	0.59
WCNP 3_14	33	0.52	0.03	0.09	0.36
WCNP 6_14	104	0.69	0.05	0.07	0.19
WCNP 7_14	73	0.63	0.08	0.04	0.25
WCNP 9_14	98	0.51	0.05	0.12	0.32
WCNP 10_14	71	0.69	0.07	0.13	0.11
WCNP_Esme 2_14	74	0.65	0.04	0	0.31
WCNP_Esme 3_14	97	0.84	0.04	0.03	0.09
Yzerfontein 2_14	79	0.71	0.10	0.04	0.15
Yzerfontein 3_14	59	0.39	0.12	0.42	0.07
Inland region					
Grassberg 1_14	41	0.49	0.29	0.05	0.17
Kloudskraal 1_14	4	0	0.50	0.5	0
Papkuilsfontein 1_14	2	0.50	0.50	0	0
Papkuilsfontein 3_14	51	0.33	0.29	0.22	0.16
Vanrhynsdorp 4_14	63	0.59	0.03	0.02	0.37
Vanrhynsdorp 5_14	31	0.77	0	0.13	0.09
Vanrhynsdorp 7_14	65	0.72	0.09	0.05	0.14

*The "Rondeberg 1_14" nest was removed from further analyses due to too few prey identified from pellets.

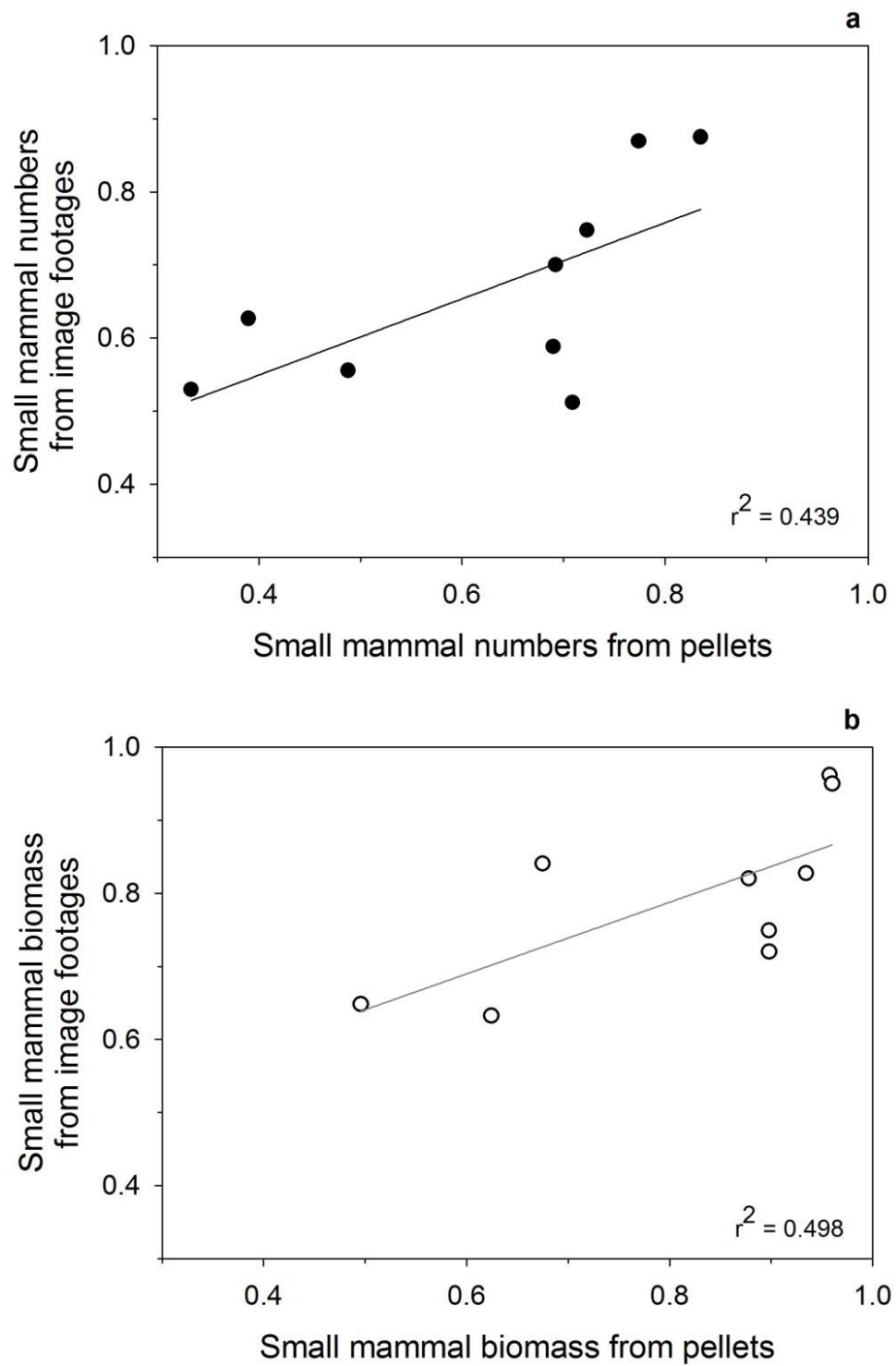


Figure B1: Relation between the proportion of (a) small mammal numbers and (b) small mammal biomass derived from image footages and in pellets. Both Pearson correlations were significant (solid lines): $r = 0.66$, $p = 0.05$ for (a) and $r = 0.71$, $p = 0.03$ for (b).

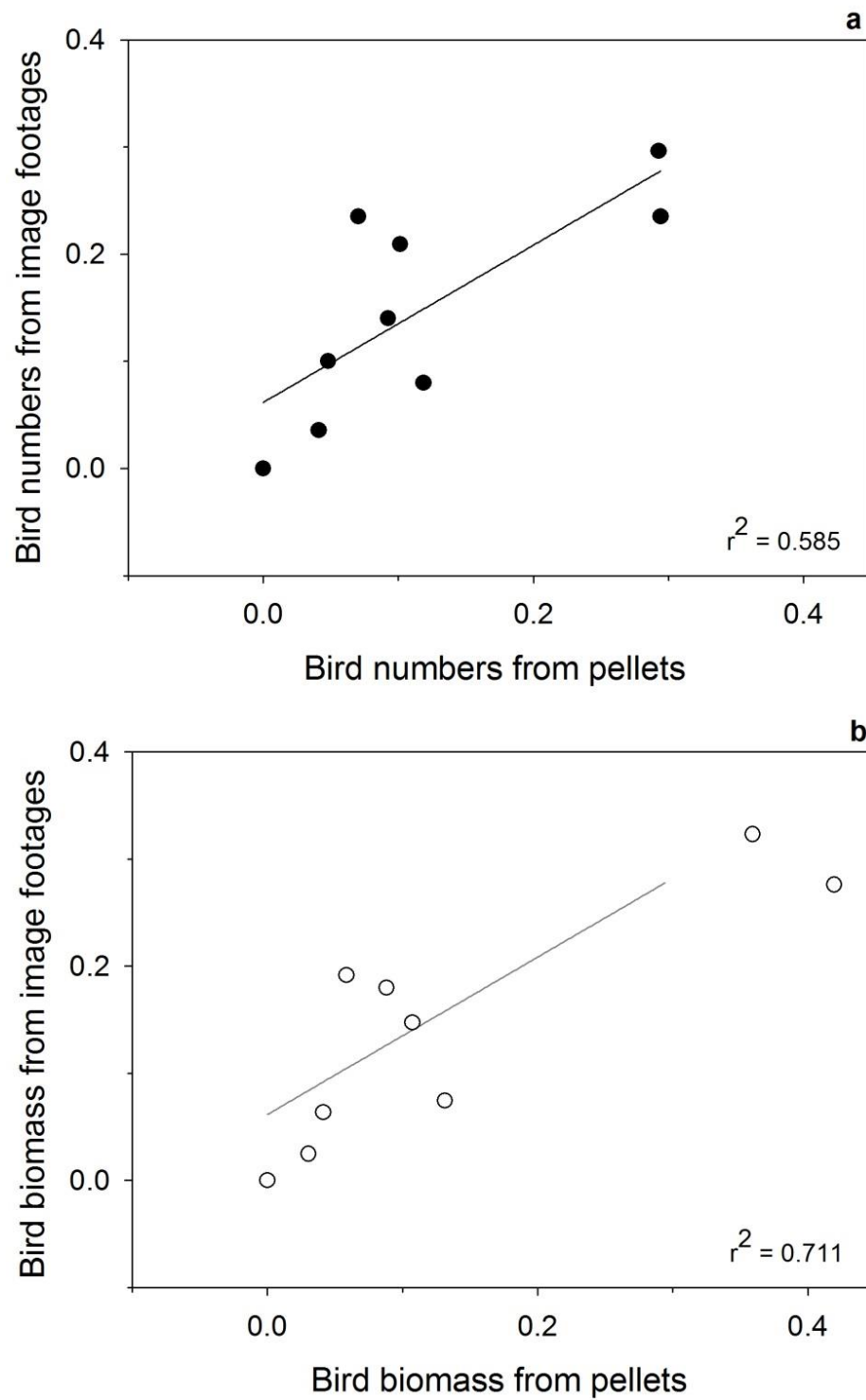


Figure B2: Relation between the proportion of (a) birds numbers and (b) bird biomass derived from image footages and in pellets. Both Pearson correlations were significant (solid lines): $r = 0.76$, $p = 0.016$ for (a) and $r = 0.84$, $p = 0.004$ for (b).

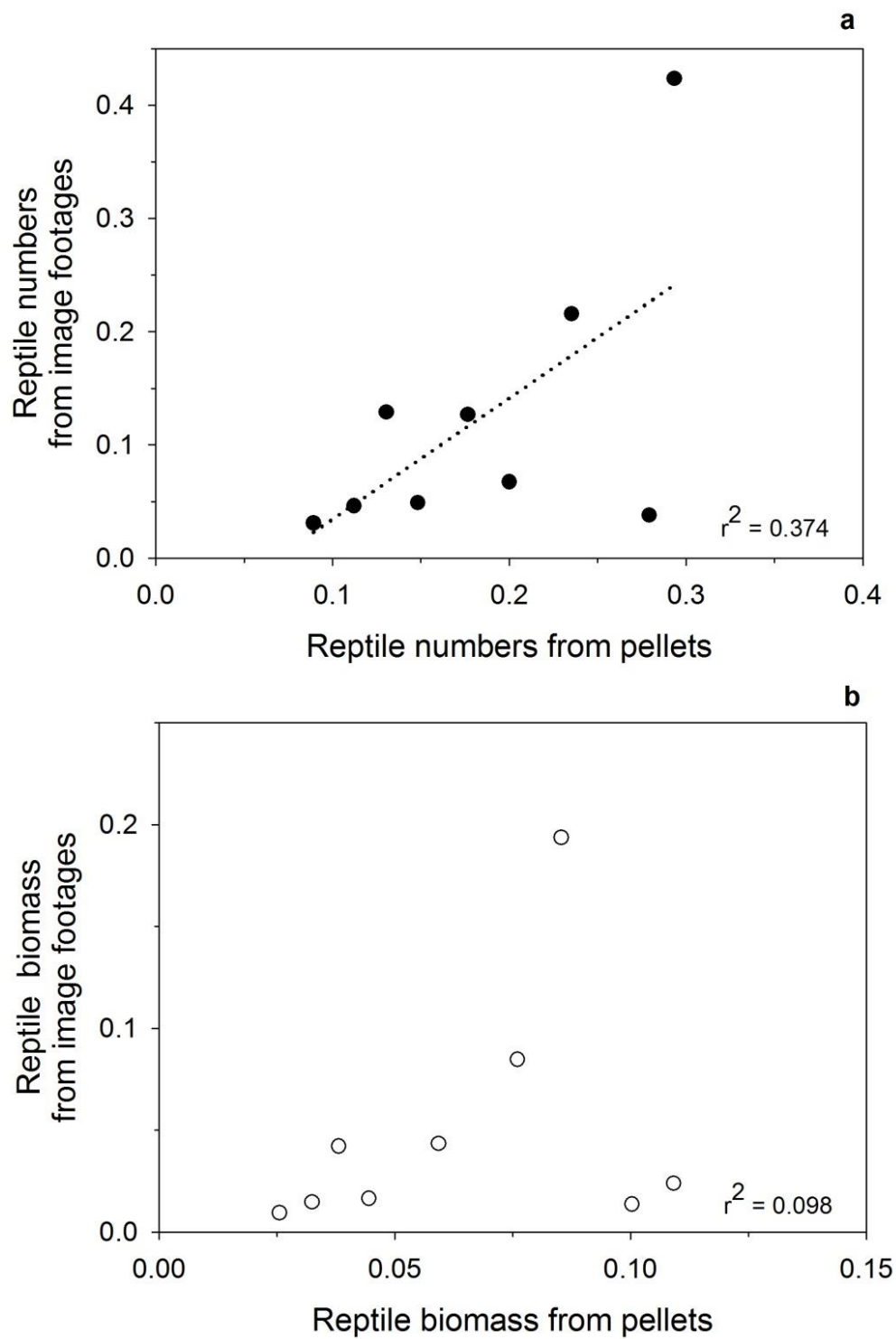


Figure B3: Relation between the proportion of (a) reptile numbers and (b) reptile biomass derived from image footages and in pellets. Pearson correlation for % of reptile was marginally significant (dashed line): $r = 0.61$, $p = 0.08$ for (a), but was not significant for reptile biomass ($r = 0.31$, $p = 0.41$) (b).

Appendix C: Regional and temporal variation of Black Harrier's diet composition at the nest level

As described in Chapter 3, it is of central importance in population ecology and wildlife conservation to understand the variations in food requirements of animals, as this helps identify where and when food may be limiting. However, the study of the temporal variation or spatial variation of a species' diet composition, at the population level, may be biased when pooling information from different nests, if there variation exists in diet among individuals. Indeed, it is well known that some breeding birds may specialize more than others in a particular type prey, and if certain individuals are sampled disproportionately in certain times or areas, this may produce skewed regional and temporal patterns in diet variation. This may arise from random individual differences or pseudo-replication of non-independent data arising from the same individual. One way to control for this is to consider only one data point per individual or pair, i.e. to conduct analyses at the nest level.

In the case of the Black Harrier population, we found a regional and temporal decline of the occurrence of small mammals in the diet of breeding birds: inland, the occurrence of small mammals decreased as the breeding season progressed (Chapter 3). In order to confirm this populational trend, we investigated the regional and temporal variation of the diet composition at the nest level.

Methods

For analyses, we selected those nests between 2006-2015 for which we had diet composition data (as described in Chapter 2) based in at least 10 identified prey from pellets (see Appendix D). Additionally, and as we had only one data point per nest, we could not assess temporal variation based on date of pellet collection. We therefore used lay date as an indicator of temporal variation. Lay date was calculated as described in Chapter 1. A total of 29 active nests ($n = 11$ inland and $n = 18$ coastal) were sampled. Number of identified prey varied from 17 to 109 prey per nest, with in average 45.3 ± 24.8 .

We analysed the proportion of small mammals in the diet using General Linear Mixed Models (GLMMs) in R v.3.2.3.. The dependent variable was a two-vector response variable (number of small mammals in the diet/number of other prey) fitted to models using a binomial error distribution and a logit link function (using the lme4 package, function glmer; Bates et al. 2012). We included region (2-level factor: coastal vs. inland), lay date (continuous variable), and the interaction region x lay date as explanatory variables. As in Chapter 3, year (7-level factor: i.e. 2007, 2008, 2011-2015) was fitted as a random term to account for potential between-year variation in diet for reasons not taken into account in our analyses (and because we were interested in describing overall patterns for any given year, rather than testing for between-year variation here).

Results

As expected, and in accordance to results shown in Chapter 3, our models showed a significant interaction region x lay date (LRT= 27.3, df= 1, $p < 0.0001$) explaining the proportion of small mammals in Black Harrier diet of individual breeding pairs. This interaction reflected a strong negative relationship between the occurrence of small mammals and the time of laying in the inland region (slope: -0.38 ± 0.06) whereas it tended to increase along the coast (slope: 0.17 ± 0.06) (Figure C1).

According to these results, we can then say that the regional and temporal variation of Black Harrier's diet composition found in Chapter 3, is the reflection of individuals breeding late in the season in the interior regions having a diet less dominated by small mammals.

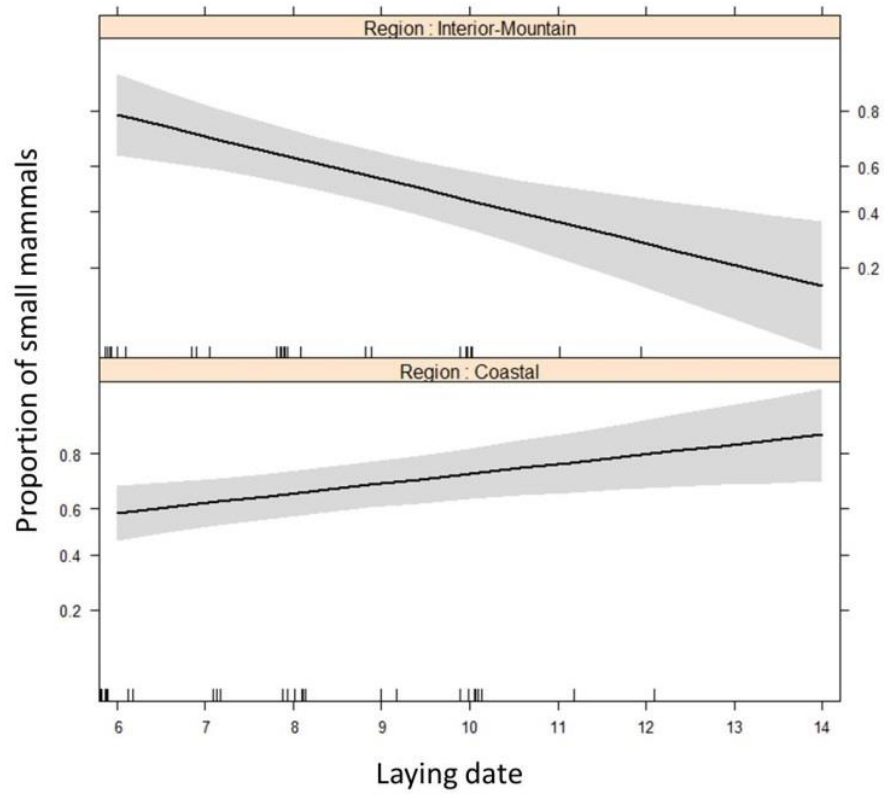


Figure C1. Results of the models testing for the effects of regional variations (coastal vs. inland) and laying date on the proportion of small mammals in Black Harrier's diet of individual breeding pairs ($n=29$).

Chapter 4

Blood concentrations of PCBs and DDTs in an avian predator endemic to southern Africa: associations with habitat, density of electric transformers and diet



While awaiting for examination of this thesis, this chapter has been sent to the journal Environmental Pollution, where it is currently under second revision: *García-Heras M.-S., Arroyo B. Simmons R.E, Camarero P.R., Mateo R., Mougeot F. Blood concentrations of PCBs and DDTs in an avian predator endemic to southern Africa: associations with habitat, density of electric transformers and diet.*

ABSTRACT

Persistent pollutants such as organochlorine compounds (OCs) have been highlighted as a cause of population decline in apex predators. Understanding the patterns of OCs contamination can be crucial for the conservation of affected species, yet little is known on these threats to African raptor species. Here we report on OC concentrations in an Endangered avian predator endemic to southern Africa, the Black Harrier *Circus maurus*. Blood samples were collected in 2012-2014 from wild nestlings (n= 90) and adults (n= 23) in south-western South Africa, where agriculture and urbanization have rapidly developed since the 1950s. Levels of polychlorinated biphenyl (Σ PCB) and dichlorodiphenyltrichloroethane (Σ DDT, for p,p'-DDT + p,p'-DDE) were detected in 79 % and 84 % of sampled individuals, respectively, with varying concentrations among demographic groups. Nestlings had significantly higher Σ PCB and p,p'-DDT concentrations than adults, which in contrast presented higher levels of p,p'-DDE than nestlings. Levels of Σ PCB significantly increased with an index of "transformer density", which combines the number and power of electricity transformers around active nests. We propose this index as a useful tool for assessing this potential source of Σ PCB exposure in wildlife. Levels of p,p'-DDE significantly increased with the proportion of wetlands within the breeding territory and with the percentage of bird biomass in the diet, confirming the intra-specific relation between diet and DDT contamination. No association was found between OC levels and the protected area status of nesting sites. We also show associations between OC levels and indicators of physiological condition. White blood cell count increased with higher p,p'-DDT levels, while the heterophils to lymphocytes ratio increased with higher Σ PCB levels, suggesting increased physiological stress and reduced immunity in contaminated individuals. Our results suggest that OCs are still a current cause of concern for endangered Black Harriers, as well as other sympatric predators. A graphical abstract representing the main results of this chapter is given in the Figure 16 below.

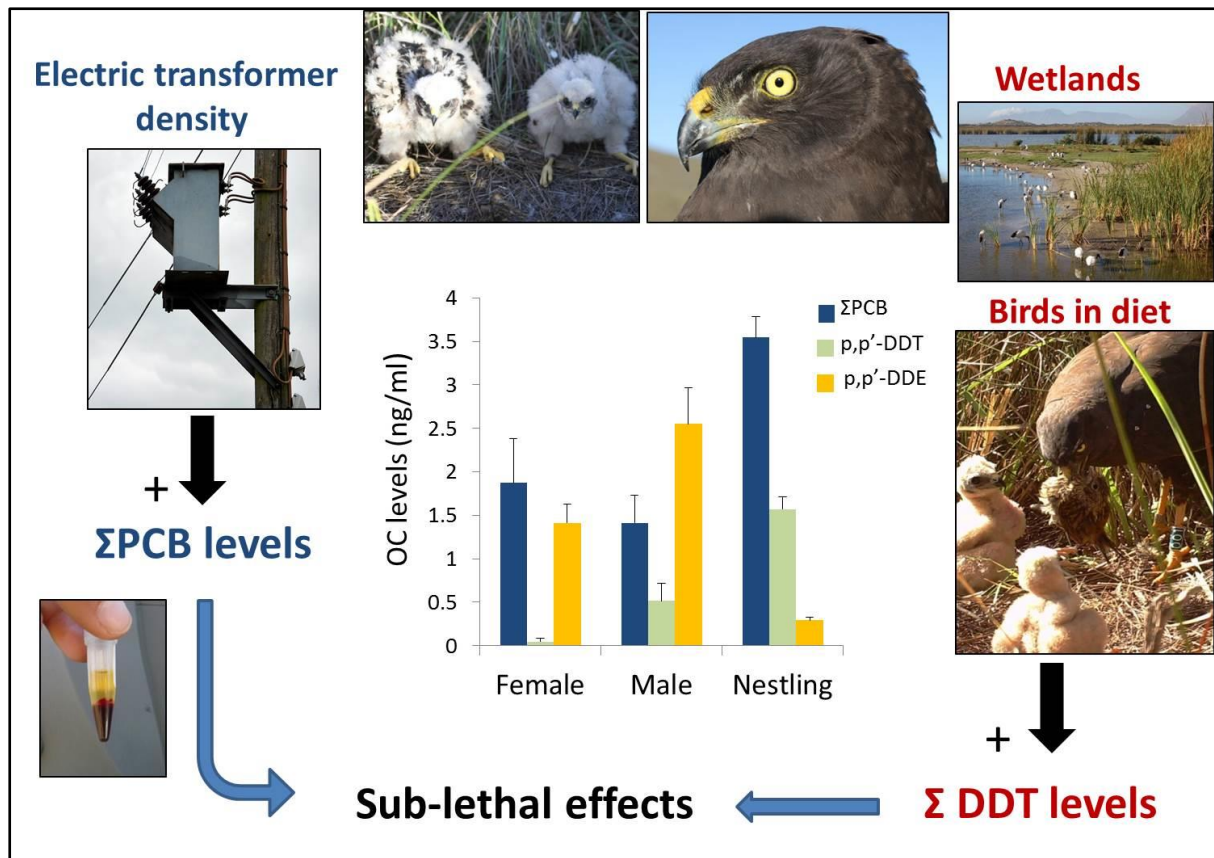


Figure 16: Graphical abstract representing the main results of Chapter 4.

INTRODUCTION

Persistent organic pollutants (POPs), such as organochlorine pesticides (DDTs and its metabolites DDEs) and industrial products such as polychlorinated biphenyls (PCBs), have been detected in all environmental ecosystems, from aquatic to terrestrial (Hoffman et al. 2003). Organochlorine compounds (OCs) are highly persistent, degrade slowly in the environment, and can affect areas far distant from their source of emission through Long Range Atmospheric Transport (LRAT) (Hoffman et al. 2003; Bustnes et al. 2004; Meijer et al. 2003; Roscales et al. 2016). This makes them highly toxic and prone to cause a number of adverse effects on wildlife and humans, even several decades after their withdrawal (Ortiz-Santaliestra et al. 2015). For instance, DDT, was widely used in agricultural areas and wetlands since its invention in the late 1940s, causing detrimental effects on the reproduction of several seabird and raptor species through embryo toxicity, eggshell thinning and breakage, that ultimately lead to the well-known declines of European and North American raptor populations (e.g. Ratcliffe 1970; Newton & Haas 1988; Newton et al. 1986; Cade & Burnham 2003). Despite its ban in the early 1970s, residues from DDT's former use are still found in the wild, with evident effects on wildlife in North America, Europe and Africa (Davies & Randall 1989; Kenntner et al. 2003; Ortiz-Santaliestra et al. 2015; Mateo et al. 2000; López-López et al. 2001; Gitahi et al. 2002; Wiesmuller et al. 2002; Wienburg & Shore 2004; Bettinetti et al. 2011). Similarly, PCBs have been extensively and widely used since the 1930s for a broad range of applications, particularly in electrical equipment (Hoffman et al. 2003), and have also been found to negatively affect wildlife (Mateo et al. 2016). PCBs were banned in most of the world at the end of the previous century (the Stockholm Convention on POPs was adopted in 2001). However, PCB contamination still persists in African countries, notably because of leakage from, or inadequate disposal of, electric transformers, continued imports of electronic waste from northern countries, shipwreck or biomass burning (Gioia et al. 2014). PCBs may therefore still represent a threat to wildlife and humans in Africa.

To help identify and reduce risks in other species including humans, many key “sentinel species” such as raptors, Polar Bears *Ursus maritimus* and whales have been studied since the last century and used as bio-indicators of pollutant contamination in the

environment (Fox 2001; Gómez-Ramírez et al. 2014). Indeed, because of their high position in trophic webs, top predators are more likely to accumulate high levels of contaminants in their tissues (e.g. liver, blood) and fat when ingesting contaminated prey, which may impact their overall health (Furness 1993; Newton et al. 1993; Ortiz-Santaliestra et al. 2015). The most affected species are those feeding on fish and birds, because of higher bio-accumulation and bio-magnification of the contaminants in their prey, and because birds of prey exhibit low enzyme activities to degrade the OCs (Furness 1993; Fossi et al. 1995; Henny et al. 2003; van Drooge et al. 2008). In this context, relating OC levels to the diet composition of a studied species is valuable for identifying sources of contamination. Additionally, relationships with habitat types present within individual breeders' territory may also hint at potential sources of contamination. For example, pesticides may be sprayed against insects in agricultural crops or against mosquitoes in wetlands, where OCs are known to bio-accumulate for years (e.g. Hoffman et al. 2003). Also, the use of toxic industrial products such as PCBs is more common in areas with high levels of urbanisation and industrialisation.

Persistent organic pollutants have been shown to cause adverse effects on a number of biomarkers of health or physiological condition in bird species (Rivera-Rodríguez & Rodríguez-Estrella 2011), including impaired immune function (Grasman et al. 1996; Bustnes et al. 2004) and increased oxidative stress (Wayland et al. 2010; Ortiz-Santaliestra et al. 2015). This may make birds more susceptible to pathogens (Grasman et al. 1996; Grasman 2002). In response to infectious organisms, bird species increase their immunity, by increasing the number of white blood cells (WBC), both heterophils and lymphocytes (Averbeck 1992). Heterophils are part of the innate immune system and provide an immediate defence by the host against infection (Roitt et al. 2001). By contrast, lymphocytes are part of the adaptive immune system and create an immunological memory after an initial response to a specific pathogen (Janeway et al. 2004). In this context, a relative increase in WBC number may be indicative of a response to infection by the immune system. The heterophils to lymphocytes ratio (H:L ratio) has also been used in birds as an indicator of physiological stress and immune response, with a high H:L ratio being indicative of increased physiological stress and reduced immunity (Siegel 1985; Ots et al. 1998; Norris & Evans 2000; Mougeot et al. 2005; Suri et al. 2016).

In southern Africa, most of the studies on OCs have been conducted either using unhatched eggs (Davies & Randall 1989; Bouwman et al. 2008, 2013, 2015) or tissues and organs collected from dead animals (van Wyk et al. 2001). Relatively little work has been published on living individuals (van Wyk et al. 2001), which may give less biased information about concentration levels in wild populations. Additionally, most studies investigating the relation between OC exposure and potential sub-lethal effects on physiological condition in raptor species have been based on experimental work with captive individuals (Bortolotti et al. 2003), often due to the difficulties of accessing nests and capturing adults in natural habitats. However, a growing number of studies highlight the importance of addressing these questions in the wild. This will allow a better understanding of the complexity of the entire system, including the relations between OC exposure, contamination by bio-accumulation and bio-magnification, the potential sub-lethal effects on individuals, and the implications for the conservation of target species (Rivera-Rodríguez & Rodríguez-Estrella 2011; Ortiz-Santaliestra et al. 2015).

The Black Harrier *Circus maurus* is a ground-nesting medium-sized bird of prey, endemic to southern Africa. Its population size has been estimated at less than 1,000 breeding individuals and the species is currently considered as Endangered in South Africa, Namibia and Lesotho (Simmons et al. 2015; Taylor 2015). This scarce raptor breeds in indigenous vegetation of south western South Africa, essentially along the coast within the Fynbos biome, and inland within the Karoo biome (Curtis et al. 2004; Curtis 2005; Chapter 1). Due to anthropogenic modification of land use during the second half of the last century, Black Harriers' natural breeding habitats have been reduced by 50 %, and many nesting areas are now surrounded by agricultural lands, sheep farming and urbanization. Breeding Black Harriers may, therefore, be currently exposed to OCs from different sources. Importantly, from 1945 until its withdrawal in the early 1980s, DDT was intensively used in South African agricultural lands, notably in maize and cotton crops, and evidence suggested that the pesticide was still used in agricultural crops after 1985 in south-western South Africa (Davies & Randall 1989; Wells & Leonard 2006), which overlaps with Black Harrier's breeding and hunting range (Curtis et al. 2004; Chapter 1). Residues from the former use of DDT may still persist in these regions and contamination of wildlife is therefore a concern. Furthermore, PCBs were still used in electrical transformers as cooling and isolating

products at least until 2010 in South Africa (Ministry of Water and Environmental Affairs, 2011). Additionally, given that Black Harriers are known to regularly consume birds, despite being small mammal specialists (Chapters 2 and 3), they may be exposed to higher OC levels as birds bio-accumulate more OCs than mammals (Fossi et al. 1995).

Because a recent study of mitochondrial DNA in adult Black Harrier feathers indicated very low genetic diversity (Fuchs et al. 2014), Black Harriers may be particularly vulnerable to negative effects of pollutants or pathogens. To date, however, there is a lack of knowledge about exposure to pollutants (specifically PCBs and DDTs) and whether these may be affecting Black Harriers.

The main goals of this study were 1) to assess the occurrence and patterns of OCs contamination in the Endangered Black Harrier population, 2) to identify correlates of OC concentrations (i.e. habitat types within the territory, electric transformer density, and diet composition) to assess sources of contaminations for Black Harriers, and 3) to assess whether the detected OCs may affect the physical (i.e. body condition index) and physiological condition (i.e. WBC number and H:L ratio) of individuals. To address these goals, we collected blood samples from wild nestling and adult Black Harriers breeding in South Africa during the 2012-2014 breeding seasons. We discuss the conservation implications for this Endangered raptor and how our results with Black Harriers may be used for a better understanding of OC exposure and its toxic effects in southern Africa, to counteract the current threats to other sympatric predators.

MATERIALS AND METHODS

Study area

Fieldwork was conducted in South Africa between July and December 2012-2014, in two main regions: along the coast of the Western Cape Province in an area north of the city of Cape Town (33.700° S, 18.45° E; 33.133° S, 18.083° E), and inland in the Northern Cape Province in the Nieuwoudtville area (31.316° S, 19.083° E). Nests were located in and around National Parks (South African National Parks –SANParks properties), Provincial Protected Reserves (Cape Nature) or on private lands (see Chapter 1 for details of nest

locations). The mild and temperate climate of the coastal region (Mucina & Rutherford 2006; Manning 2007; Chapter 1) has contributed to a rapid development of cereal agriculture, viticulture and urbanization in this region. This includes human population expansion to 4 million inhabitants and the presence of the only nuclear power station in Africa since the 1950s. Outside this urbanized environment, large tracts of natural vegetation still remain, mainly in protected areas (Curtis et al. 2004; Chapter 1). By contrast, the inland region is more rural, sparsely populated (i.e. < 15,000 inhabitants) with an old and widespread tradition of agricultural lands and sheep farming, where natural vegetation is highly fragmented (Reyers et al. 2009).

Habitat parameters

Adults or nestlings from a total of 49 nests were monitored for OC levels during the study period. Coordinates for these 49 nests were incorporated in a geographical information system (QGIS Valmiera 2.2.0), projected onto WGS84-UTM-34S coordinate reference system. Using the GIS, we first created a 5 km radius buffer around each nest, hereafter referred to as the “breeding territory”. This corresponds to the average home range of an individual, as estimated from data from 12 GPS-tagged adult Black Harriers (RES *unpublished data*). Within this buffer we identified and calculated for each nest the following three variables as potential sources of OC exposure for Black Harriers: i) Proportion of agricultural land cover, i.e. potential source of pesticide contamination; ii) Proportion of wetland cover as a potential source of OCs contamination by bioaccumulation in the sediments; iii) An index of the density of electric transformers (hereafter “Transformer Density Index”) as a measure of potential sources of PCBs in the environment. PCBs have been widely used as dielectric fluids in electrical transformers and capacitors since 1930 (Hoffman et al. 2003). Our index was based on the number and power of electricity transformers (Trf) within the 5 km buffer area around study nests. It was calculated as the sum of the kilovolt-ampere or kVA-rating of all the transformers within the 5km buffer area, divided by the land surface area (kVA/km^2). The Transformer Density Index ranged from 0 to 227.9 kVA/km^2 depending on nesting sites and averaged 18.7 Trf/ km^2 . The land use data were obtained from the South African National Land Cover Map (NLC) 2014. The electric transformer data were obtained from the Electricity Supply Commission of South Africa (Eskom’s) 2014 shapefiles in GIS.

Finally, each nest was attributed a “protected area status” to test for possible differences in OC levels in and outside protected areas. Nests located inside national parks or natural reserves were considered as “protected” (n= 21), whereas all others were considered “not protected” (n= 28).

Dietary assessment

We estimated diet composition at each of the 49 monitored nests through the analysis of regurgitated pellets collected at monitored nests during the breeding season from August to December. Pellet contents including prey remains such as bones, scales, feathers or hairs, were analysed and identified following the same methods described in Chapter 2. Additionally, in 2014, cameras were set at 18 active nests during the nestling period, i.e. when chicks were 7-41 days old. Cameras were programmed to take a picture every 5 seconds, or to record a 60 seconds video sequence (1 second between two videos), and were set from sunrise until sunset e.g. 06h00 – 19h59. We obtained a total of 1557 videos and 90,692 minutes of recording time averaging 527 ± 40.2 min per nest (Chapter 3 for further details). Images and video footage were analysed to identify the type of prey delivered, categorized as small mammal, bird, reptile or unidentified prey item. Data on prey types and cameras were combined to identify the percentage of each prey type among all identified prey items for each monitored nest. The number of identified prey per nests varied from 1 to 107; A bootstrapping analyses (Appendix D) indicated that a minimum of 10 identified prey was needed to obtain an unbiased estimate of diet composition. We used this as our minimum sample because both the average values of the proportion of each prey type and their standard deviations converged from a minimum of 10 identified prey. This pared our sample down to 30 nests with at least 10 identified prey to estimate diet composition. Among those nest, the average number of identified prey was 48.4 ± 0.18 . Thereafter, the percentage of biomass of each category of prey was estimated as described in Chapter 2. The contribution of reptiles to consumed biomass among the three prey types was only ca. 5 % and thus appeared negligible. The proportion of bird biomass was negatively related to proportion of small mammal biomass (Pearson correlation: $r = -0.98$, $p <$

0.0001, $n = 109$). Therefore, in order to simplify the analyses, we only used the proportion of bird biomass as indicator of diet composition.

As with other raptor species, female Black Harriers take care of nestlings at the nest and performs all the brooding while the male captures and provides the food in the early nestling period (Simmons 2000; Redpath et al. 2002a). Therefore, females and nestling feed on the same prey that is provided by males, and we assume that that males' diet composition is likely to be similar to the one he provides to the nest. We therefore attributed the same diet composition to all members of a given sampled nest.

Field procedures and sample collection

Once nestlings were 15-39 days old using ageing criteria (Appendix A), we attempted capturing both adult breeders using a Dho Gaza net and a stuffed Spotted Eagle Owl; these were set at a distance of 20-30 m from the nest to simulate a predator intrusion and elicit attacks from the adults defending the nestlings (Appendix E). After capture, adults and nestlings were weighed and measured and individually marked with a metal and a coloured ring with a unique alpha-numeric code. Tarsus length to the nearest 0.1 mm (using an electronic calliper), and body mass to the nearest 5-10 g (using a pesola) were measured, and we calculated a body condition index using the residuals from the relation between the body mass and the age of nestlings for each sex separately (see Appendix A). For adults, the body condition index was calculated using the weight relative to the tarsus length, as an indicator of size.

Blood samples were taken to determine (i) Organochlorine Compounds (OCs) contamination, (ii) white blood cell counts and (iii) sex using DNA analysis. Each blood sample of 0.7-1 ml was collected from the brachial vein using a heparinized syringe. A drop of fresh blood was deposited and smeared on a glass slide, before being fixed in methanol and dried. The rest of the blood was kept in a heparinized Eppendorf vial in a polystyrene cool box filled with ice blocks. Within 30-40 min after collection, the samples were centrifuged for 15 min using a Ministar portable centrifuge (VWR, Radnor, Pennsylvania) to separate the plasma from the red cells (i.e. hereafter "blood pellet"). Both set of samples were immediately placed in a portable freezer, and frozen at -80°C on arrival at the lab from

the field (< 3 hours after collection) until analyses. Plasma samples were used to quantify the concentrations of OCs. While adult harriers were sexed morphometrically (Simmons et al. 2005), nestling's were sexed genetically using DNA analysis of the blood pellet (see below).

After sampling, nestlings were replaced at the nest, and adults were freed at their place of capture within approximately 20 min. A total of 90 nestlings (n= 40 males, n= 50 females), and 23 adults (15 females and 9 males, among which 7 were breeding pairs) were sampled. The field work protocols were approved by the University of Cape Town's science Faculty Animal Ethics Committee, Permit number: A1/2014/2013/V21/GC.

DNA-extraction protocol and molecular sexing method

We lysed blood cells at 55° C for 10 h in 250 µl extraction buffer (0.01 mM Tris-HCl pH= 8.5; 0.01 mM NaCl; 0.05 mM EDTA pH= 8.0, 2 µl of SDS (20 %), 8 µl Proteinase K (10 mg/ml). We used differential precipitation with NH₄Ac (4M, pH= 7.5) and 99 % ethanol to separate DNA from proteins. Samples were finally diluted in ddH₂O to a working DNA concentration of 25 ng/ml.

DNA from the sex chromosomes (Z and W) was amplified by PCR using the primers 0057F and 002R (Round et al. 2007). Each reaction included approximately 50 ng of genomic DNA. All reactions were performed in 10-µl volumes containing 0.25 U of Taq DNA polymerase (Biotools), 0.125 mM of dNTP's, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3.0 mM MgCl₂ and 4 pmol of each primer. The thermal profile consisted of an initial denaturing step at 94°C for 3 min, following by 30 cycles of (30 s at 94°C, 45 s at 50°C and 45 s at 72°C), and a final step at 72°C for 10 min. We evaluated 2.5 µl of each reaction on a 2 % agarose gel and using 0.5 x TAE buffer, using 100 bp DNA ladder as reference (Biotools). We routinely used negative controls (samples with ddH₂O instead of genomic DNA as template), and positive controls (genomic DNA from adult male and female Montagu's Harrier), to ascertain that the outcome of each PCR run was not affected by contamination. All necessary precautions were used to prevent PCR contaminations.

Organochlorine compounds analyses

Concentrations of PCBs and organochlorine pesticides were determined in plasma samples following the method previously described and validated in Mateo et al. (2012). This method is based on the extraction of plasma samples with n-hexane and the clean-up of the extract with sulfuric acid. Organochlorine concentrations were measured by gas chromatography coupled to an electron capture detector (GC-ECD) equipped with a column HP-5 30 m, 0.32 mm, 0.25 μ m both purchased from Agilent Technologies. Pesticide-Mix 13 (Dr. Ehrenstorfer standard) containing *cis*-chlordane, *trans*-chlordane, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, α -endosulfan, β -endosulfan, HCB, α -HCH, β -HCH, γ -HCH, δ -HCH, ϵ -HCH, heptachlor, heptachlor-exo-epoxide, methoxychlor, and PCBs 28, 52, 101, 138, 153 and 180 was used for calibration purposes. Recoveries of the analysed compounds were calculated with plasma samples of farm reared Red-Legged Partridges *Alectoris rufa* spiked with 2.5, 5 or 10 ng/ml (n= 5 for each level). Except for some cyclodienes and methoxychlor that are completely lost in the clean-up step, most of the recoveries of the analysed compounds were above 70 % and those detected in the Black Harriers all showed recoveries around 100 % (Appendix F). OCs levels are expressed in ng/ml and we used the mean value \pm standard deviation (SD) to describe the data. Overall, OC levels were determined for 23 adults and 90 nestlings.

Blood smear analyses

After being fixed with ethanol, blood smears were stained with the May-Grünwald-Giemsa method. To determine the white blood cell (WBC) count, all smears were inspected under a microscope at 1,000x magnification with an oil immersion by the same experienced person, who counted the number of leucocytes found in 10,000 blood cells from a randomly chosen area central on the smear. A high WBC count (number of leucocytes / 10,000 cells) may be indicative of an increased circulation of leucocytes because of an infection (Bustnes et al. 2004; Norris & Evans 2000). In addition, a total of 100 leucocytes were classified as lymphocytes, monocytes, eosinophils, heterophils and basophils. We calculated the ratio of heterophils to the lymphocytes (H:L ratio) for each individual, which may be indicative of an increasing physiological stress and a reduced immunity (Siegel 1985; Ots et al. 1998;

Mougeot et al. 2005). Overall, WBC and H:L ratio were determined for 23 adults and 88 nestlings.

Statistical analyses

All statistical analyses were conducted using R 3.2.3 (the R Foundation for statistical computing, 2015).

We first looked for general patterns in variation of OC levels. For this, we conducted General Linear Mixed Models (GLMMs), where the log transformed response variables (Σ PCB, Σ DDT, p,p'-DDT and p,p'-DDE) were fitted to models using a normal distribution (package lme4, function lmer and a logit function; Bates et al. 2012). Nest was included as a random effect in all models to take into account the non-independence of samples coming from the same nest.

We first checked for differences among demographic groups (3-level factor: adult females, adult males and nestlings) and years (3-level factor: 2012, 2013, 2014) on OC levels. Pairwise comparisons using Tukey tests were also conducted to compare the significance among demographic groups, two by two. For this model, analyses were conducted on 90 nestlings, 15 adult females and 9 adult males. We then tested for nestlings only, the effects of year, age, sex and the interaction age \times sex to identify the possible temporal acquisition of OCs, i.e. early or later during the nestlings' growth.

We subsequently investigated the relation between OC levels and habitat variables. Specifically, for the Σ PCB we included the following explanatory variables: proportion of wetland area in the breeding territory, protected area status (2-level factors: protected vs. not protected), transformer density index, demographic group (2-level factor: adults vs. nestlings), and the interactions between demographic group and transformer density index, and between demographic group and wetlands. For the Σ DDT, the p,p'-DDT and the p,p'-DDE levels, we included the following as explanatory variables: proportion of wetland, proportion of agricultural cover, protected areas, demographic group, and the interactions between demographic groups and wetlands and agricultural cover.

Third, after taking into account the effects of significant habitat variables, we also tested for an additional effect of the diet composition on OC levels. For this, we included the percentage of bird biomass in the diet as a further explanatory variable in the models. These models were performed using 56 nestlings, 11 adult females and 9 adult males, for which diet data were available.

Finally, to investigate whether contaminants influenced Black Harrier physical or physiological condition we fitted the body condition index, the H:L ratio (square root transformed), and the WBC ratio (log transformed) as response variables to models, using a normal distribution (package lme4, function lmer and a logit function; Bates et al. 2012). As nestlings and adults have different physiological metabolisms, we analysed each group separately. Specifically, for nestlings we first looked at the effects of year, day of sampling, and sex; subsequently, after accounting for significant variables, we looked at the additional effect of the OC variables (Σ PCB, Σ DDT, p,p'-DDT, p,p'-DDE). Nest was kept as a random term in these models to take into account differences between siblings. For adults, models were performed fitting only the OC variables and sex as explanatory variables.

Type III results are presented. Significance of interactions were tested using the function drop 1 in R (stepwise backward selection). Non-significant interactions and factors were excluded from final models.

RESULTS

Occurrence and levels of organochlorine compounds in adults and nestlings

A summary of the detected OCs and their levels in the blood plasma of sampled Black Harriers is given in Table 9.

Overall, 79 % of sampled individuals (n= 114) had detectable plasmatic PCBs levels (PCB congeners #52, 101, 153, 138 and 180) (Table 9). The sum of the concentrations of all detected PCBs (Σ PCB) did not differ between years ($\chi^2 = 1.60$, d.f.= 2, p= 0.45), but differed significantly among demographic groups ($\chi^2 = 9.81$, d.f.= 2, p= 0.007; Figure 17a). Pairwise

comparisons using Tukey tests indicated that nestlings had significantly more Σ PCB than adult females, while adult males had intermediate levels (Figure 17a, Table 9).

Table 9. Mean (\pm SD) blood plasma concentrations (ng/ml) of Organochlorine Compounds (OCs) in the Black Harriers from South Africa, 2012-2014. Data ranges [min-max] are given in brackets. Sample size refers to number of individuals. The detection limit was 0.01 ng/ml. Σ PCB is sum of the concentration of all PCB congeners. Σ DDT is the sum of the concentration of p,p'-DDT and p,p'-DDE.

	Adult Females	Adult Males	Nestlings*
PCB 52	1.14 \pm 1.57 [0 – 4.40]	0.64 \pm 0.83 [0 – 1.92]	2.65 \pm 2.71 [0 – 11.44]
PCB 101	0.40 \pm 0.93 [0 – 3.61]	0.39 \pm 0.50 [0 – 1.34]	0
PCB 138	0.02 \pm 0.07 [0 – 0.25]	0	0.01 \pm 0.09 [0 – 0.59]
PCB 153	0.11 \pm 0.27 [0 – 0.98]	0.13 \pm 0.28 [0 – 0.82]	0.37 \pm 0.65 [0 – 4.03]
PCB 180	0.21 \pm 0.28 [0 – 0.72]	0.25 \pm 0.30 [0 – 0.64]	0.52 \pm 1.19 [0 – 5.10]
Σ PCB	1.87 \pm 2.32 [0 – 6.93]	1.41 \pm 1.16 [0 – 3.23]	3.55 \pm 3.06 [0 – 13.74]
p,p'-DDT	0.05 \pm 0.18 [0 – 0.71]	0.51 \pm 0.69 [0 – 2.14]	1.57 \pm 1.88 [0 – 9.84]
p,p'-DDE	1.41 \pm 0.97 [0 – 3.38]	2.55 \pm 1.51 [1.05 – 4.89]	0.29 \pm 0.48 [0 – 2.21]
Σ DDT	1.46 \pm 0.99 [0 – 3.38]	3.06 \pm 1.50 [1.34 – 5.89]	1.86 \pm 1.96 [0 – 9.84]
Sample size	15	9	90

* OC levels did not vary significantly according nestling sex (see results) so data have been pooled for all nestlings.

We detected DDTs in the plasma of 84 % of sampled individuals (n= 114), including one DDT isoform (p,p'-DDT in 53 % of sampled individuals) and two metabolites (o,p'-DDE in 0.9 % of individuals and p,p'-DDE in 49 % of sampled individuals; Table 9). However, o,p'-DDE was only found in one sampled nestling, so we only considered p,p'-DDE levels for subsequent analyses. Considering the overall sum of p,p'-DDT and p,p'-DDE (Σ DDT) levels, we found that concentration did not differ between years ($\chi^2 = 2.36$, d.f.= 2, p= 0.31), but

differed significantly among demographic groups ($\chi^2 = 7.40$, d.f. = 2, $p = 0.025$). Pairwise comparisons showed that adult males had significantly more Σ DDT than nestlings, and adult females had intermediate levels which were significantly different from neither adult males nor nestlings (Figure 17b, Table 9). We further investigated variation in p,p'-DDT and p,p'-DDE levels separately. The p,p'-DDT levels did not differ between years ($\chi^2 = 0.12$, d.f. = 2, $p = 0.94$), but again differed among demographic groups ($\chi^2 = 18.29$, d.f. = 12, $p < 0.001$). Nestlings had the highest p,p'-DDT levels (1.57 ± 1.88) followed by adult males (0.51 ± 0.69), and adult females had significantly lower values than nestlings (0.05 ± 0.18 ; Figure 17c). We found no significant variation in p,p'-DDE levels between years ($\chi^2 = 3.78$, d.f. = 2, $p = 0.15$), but marked differences among demographic groups ($\chi^2 = 134.32$, d.f. = 2, $p < 0.001$). For this metabolite, the lowest levels were found in nestlings (0.29 ± 0.48 , significantly lower than those of adults; Figure 17d). In addition, adult males (2.55 ± 1.51) had significantly higher p,p'-DDE levels than adult females (1.41 ± 0.97 , Figure 17d).

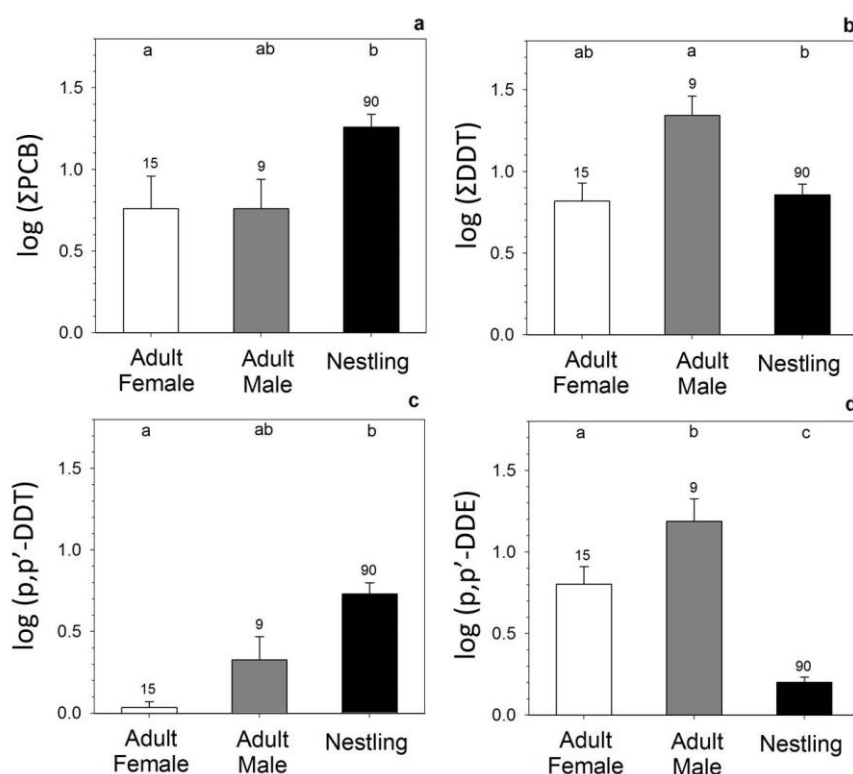


Figure 17. Mean (\pm SE) blood plasma concentrations (log transformed values) of **a)** Σ PCB, **b)** Σ DDT, **c)** p,p'-DDT, and **d)** p,p'-DDE according to demographic group (adult females: white bars; adult males: dark grey bars; nestlings: black bars). For pairwise comparisons, different letters above bars indicate significant differences ($p < 0.05$) between demographic groups (Tukey's tests). Sample sizes (number of individuals sampled in each group) are indicated above the error bars.

Territory characteristics and organochlorine compound levels

After taking into account differences in Σ PCB levels between adults and nestlings ($\chi^2 = 7.04$, d.f. = 1, $p = 0.008$), we found no evidence for Σ PCB levels to vary with the proportion of wetlands in the breeding territory ($\chi^2 = 0.70$, d.f. = 1, $p = 0.40$) or the protected area status ($\chi^2 = 0.04$, d.f. = 1, $p = 0.84$). However, we found a significant positive relationship with the Transformer Density Index ($\chi^2 = 5.20$, d.f. = 1, $p = 0.023$; Figure 18) in both adults and nestlings. There was no significant interaction between demographic group and Transformer Density Index ($\chi^2 = 0.17$, d.f. = 1, $p = 0.68$; Figure 18).

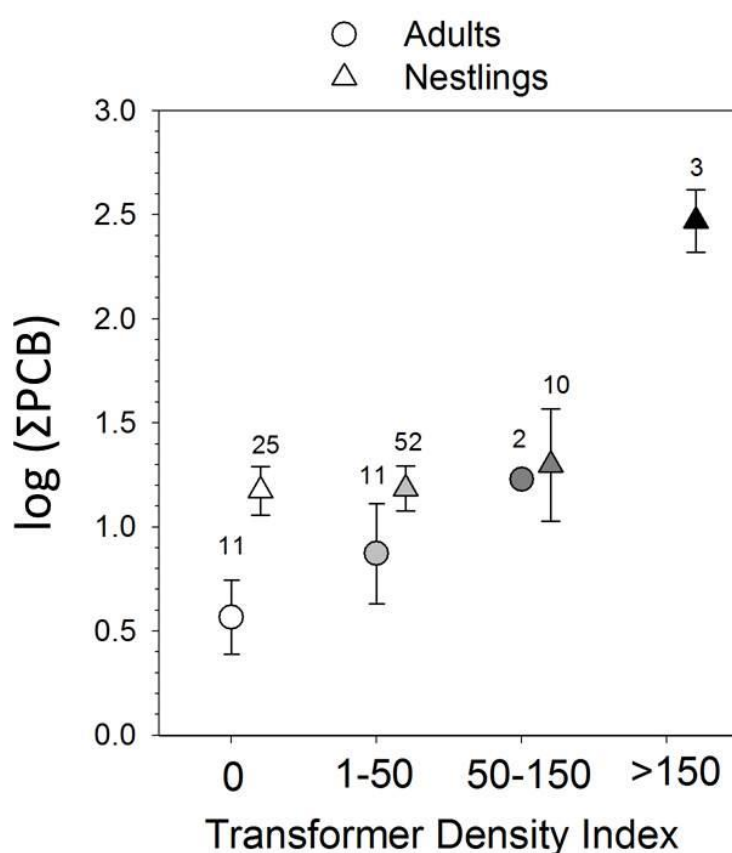


Figure 18. Mean (\pm SD) blood plasma concentrations of PCB (ng/ml, log-transformed) according to an index of transformer density within the breeding territory (overall kVA-rating per km²). For illustration purpose, the transformer index has been categorized (four classes: white: 0, light grey: 1-50, dark grey: 50-150, black: >150). Data are shown by demographic group (adults: circles; nestlings: triangles) separately. Numbers above the error bars refer to number of individuals.

After accounting for differences in Σ DDT levels between demographic groups ($\chi^2=7.91$, d.f.=1, $p=0.019$), we found no significant effect of agriculture cover ($\chi^2=0.791$, d.f.=1, $p=0.37$) or protected area status ($\chi^2=0.01$, d.f.=1, $p=0.99$), but we found a significant and positive effect of the proportion of wetlands on Σ DDT levels ($\chi^2=6.18$, d.f.=1, $p=0.013$; slope \pm SE: 0.21 ± 0.085). This effect was similar for adult males, adult females and nestlings. There was a non-significant interaction between demographic group and wetland cover: $\chi^2=0.38$, d.f.=2, $p=0.83$. When considering p,p'-DDT and p,p'-DDE levels separately, we found that the effect of the proportion of wetland was only significant for p,p'-DDE levels ($\chi^2=11.62$, d.f.=1, $p<0.001$), not for p,p'-DDT alone ($\chi^2=0.304$, d.f.=1, $p=0.58$).

Diet composition and organochlorine compound levels

For the sub-sample of 29 nests for which we had detailed information on prey consumed, after controlling for differences among demographic groups and the effect of transformer density, we found no significant association between Σ PCB levels and diet composition (% birds: $\chi^2=0.77$, d.f.=1, $p=0.38$). For Σ DDT levels, however, we found that, controlling for differences among demographic groups and the effect of wetland cover, Σ DDT levels increased significantly with the percentage of bird biomass consumed ($\chi^2=5.84$, d.f.=1, $p=0.016$; slope \pm SE: 0.996 ± 0.413).

Considering p,p'-DDT and p,p'-DDE levels separately, we found no associations between p,p'-DDT levels and the percentage of bird biomass ($\chi^2=0.16$, d.f.=1, $p=0.69$). By contrast, we found a strong positive association between p,p'-DDE levels and the proportion of consumed bird biomass ($\chi^2=12.41$, d.f.=1, $p<0.001$; slope: 0.979 ± 0.278 ; Figure 19).

Physical condition and organochlorine compound levels

We found no significant associations between nestling condition index and Σ PCB or Σ DDT levels (Σ PCB: $\chi^2=0.024$, d.f.=1, $p=0.877$; Σ DDT: $\chi^2=1.01$, d.f.=1, $p=0.314$), or between adult condition index and OC levels (Σ PCB: $\chi^2=0.70$, d.f.=1, $p=0.40$; Σ DDT: $\chi^2=0.20$, d.f.=1, $p=0.65$). We thus had no evidence that contaminated harriers are relatively lighter than contaminant free ones.

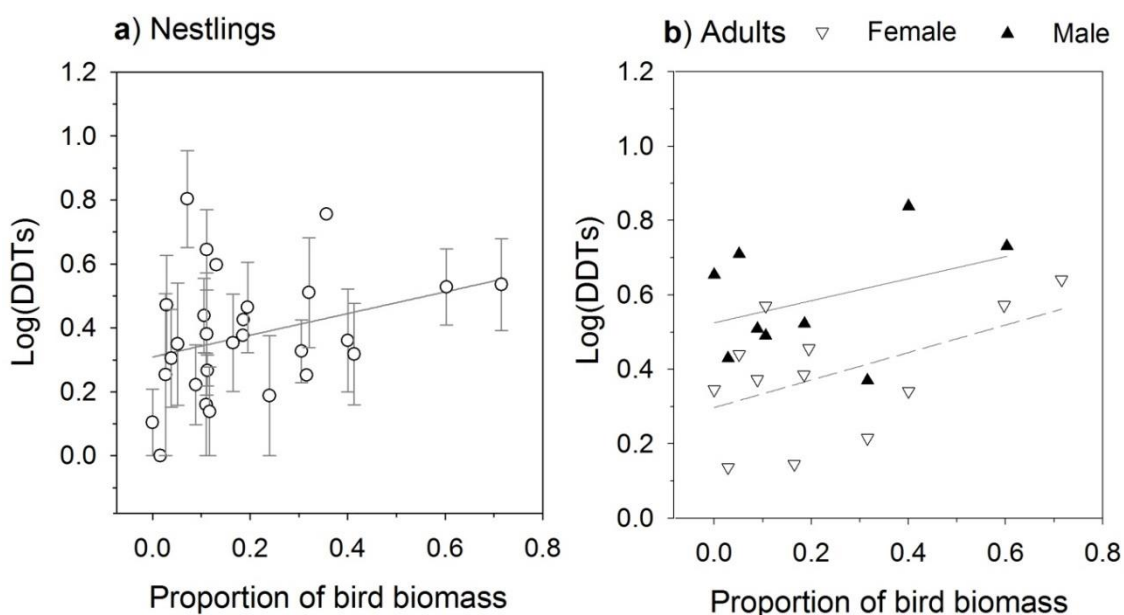


Figure 19. Relationship between plasma Σ DDT concentration (log transformed; ng/ml) and the proportion of bird biomass consumed by **a)** nestling and **b)** adult Black Harriers.

WBC count, H:L ratio and organochlorine compound levels

Among nestlings, the WBC count did not vary with Σ PCB levels ($\chi^2 = 2.3589$, d.f. = 1, $p = 0.12$), but tended to increase with Σ DDT levels ($\chi^2 = 3.795$, d.f. = 1, $p = 0.051$; slope: 0.13 ± 0.065). The positive association was between WBC count and p,p'-DDT levels ($\chi^2 = 3.08$, d.f. = 1, $p = 0.080$; slope: 0.11 ± 0.063) rather than between WBC count and p,p'-DDE levels ($\chi^2 = 0.58$, d.f. = 1, $p = 0.445$). In adult birds, we found that WBC count was greater in females than in males ($\chi^2 = 4.25$, d.f. = 1, $p = 0.039$; LS means \pm SE: 4.35 ± 0.11 and 4.03 ± 0.14 , respectively), but did not vary with either Σ PCB levels ($\chi^2 = 0.10$, d.f. = 1, $p = 0.75$) or with Σ DDT levels ($\chi^2 = 0.03$, d.f. = 1, $p = 0.85$).

Considering the H:L ratio, we found a positive association with Σ PCB levels in both nestlings ($\chi^2 = 3.48$, d.f. = 1, $p = 0.06$; slope: 0.066 ± 0.036) and adults ($\chi^2 = 6.25$, d.f. = 1, $p = 0.012$; slope: 0.050 ± 0.021 ; Figure 20). We found no significant association between H:L ratios and Σ DDT levels in either nestling ($\chi^2 = 0.82$, d.f. = 1, $p = 0.36$) or adult birds ($\chi^2 = 2.20$, d.f. = 1, $p = 0.14$).

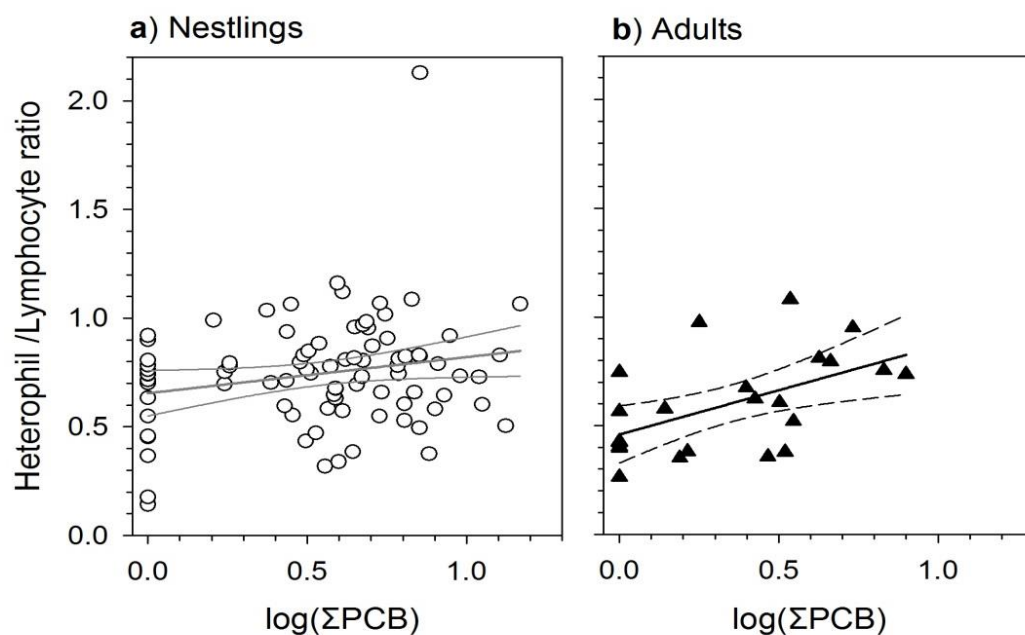


Figure 20. Relationship between the Heterophils : Lymphocytes ratio (squared root transformed) and plasma levels of Σ PCB (ng/ml, log-transformed values) in **a)** nestling and **b)** adult Black Harriers. Linear fit (solid line) and \pm 95 % confidence intervals (dashed line) are showed in dark grey for nestlings and in black for adults.

DISCUSSION

This study represents one of the few assessing the presence of organochlorine compounds (OCs) in a southern African raptor using blood samples from wild and living individuals (van Wyk et al. 2001). Furthermore, it highlights associations between OC levels, habitat types, diet composition and indicators of physiological condition that help understand exposure routes and potential sub-lethal effects. To the best of our knowledge, this study is one of the very few that assessed OC variations in harrier species (Pain et al. 1999). Σ PCB and Σ DDT (p,p'-DDT + p,p'-DDE) levels were detected in 79 % and 84 % of our sampled individuals, respectively, suggesting that environmental contamination is relatively widespread within our study regions. Detected levels were relatively low, but within the range or slightly higher than those found in recent northern-hemisphere studies of other raptors (e.g. Sonne et al., 2012; Bustnes et al. 2013; Eulaers et al., 2014; Gómez-Ramírez et al. 2014; Ortiz-Santaliestra et al. 2015), for which adverse physiological effects have also been reported (e.g. Sonne et al. 2012; Ortiz-Santaliestra et al. 2015). Therefore, the detected levels in Black Harriers seem

biologically relevant and may have conservation implications for this scarce and endangered species, as well as other sympatric predators.

Industrial activity has been associated with contamination by PCBs in raptor species around the world, with higher levels in individuals inhabiting more industrialized areas (e.g. Naso et al. 2003; Bowerman et al. 2003; Rivera-Rodríguez & Rodríguez-Estrella 2011; Gómez-Ramírez et al. 2014; Ortiz-Santaliestra et al. 2015). Unexpectedly, our results showed higher Σ PCB levels in nestling than adult female Black Harriers, while adult males had intermediate levels. Taking into account that the accumulation of OCs in bird tissues occurs over time, i.e. intake rate exceeds excretion rate (Kenntner et al. 2003; Goutner et al. 2011), it is usually expected that adults will have higher blood levels of OCs than nestlings, as described in several species of Vulture (Goutner et al. 2011), and Bonelli's Eagle *Aquila fasciata* (Ortiz-Santaliestra et al. 2015). One explanation why nestling harriers have higher Σ PCB concentrations than adults may arise from differences in the P450-system between nestlings and adults. The P450-system is responsible for the bio-transformation of PCBs, and it is possible that nestling Black Harriers may have a low P450-associated enzyme activity and consequently a lower capacity for bio-transforming PCBs than adults (Rattner et al. 1997; Jenssen et al. 2001; Frank et al. 2001; Naso et al. 2003). Furthermore, our results show that Σ PCB levels tended to decrease with increasing nestling's age, supporting the latter explanation. Another perplexing result is that the high levels of Σ PCB came essentially from higher levels of the congener PCB 52. Because of its lower degree of chlorination, the PCB 52 is usually more rapidly metabolized and excreted than congeners with a higher degree of chlorination such as PCB 138, 153, 180 (Oliver & Niimi 1988; Walker 2008; Naso et al. 2003), and our results are therefore unexpected. The latter may be explained by a recent ingestion of contaminated prey. Indeed, Olsson et al. (2000) demonstrated that blood Σ PCB levels in raptors may vary with recent feeding and especially for those congeners that are quickly metabolized such as PCB 28, 31 and 52. This also appeared to be the case in a recent study on nestling Bonelli's Eagles from Spain (Ortiz-Santaliestra et al. 2015). In accordance with the Stockholm convention that aimed to restrict and eliminate the production and use of persistent organic pollutants, the use of less toxic, lower-chlorinated congeners may have been prioritized over the use of the more toxic higher-chlorinated congeners (Georgii et al. 1994; Ministry of Water and Environmental Affairs 2011). This may also explain the higher

levels of the PCB 52 congeners in Black Harrier's blood. This appeared to be the case in Germany, where sampled Red Foxes (*Vulpes vulpes*) presented higher concentrations of lower-chlorinated congeners (PCBs 24, 49 and 52 vs. PCBs 138, 153, and 180) in their body fat (Georgii et al. 1994). However, no association between Σ PCB levels and the proportion of avian prey biomass was found, suggesting that the Σ PCB contamination in Black Harriers comes from all types of prey similarly, or from other sources.

PCBs are not produced in South Africa, but they have been imported in large quantities since the 1930s mainly to be used in electricity generating equipment (Ministry of Water and Environmental Affairs, 2011). Worldwide PCB production was stopped in 1989, although in South Africa, PCB oils were used in electric transformers and capacitors until at least 2010. According to the Ministry of Water and Environmental Affairs (2011), 32% of all transformers had a PCB content of 1-19 ppm, 2% had 20-49 ppm, 62% had 50-499 ppm, and 4% had a PCB content greater than 500 ppm. In our study, we found a significant association between Σ PCB levels in adult and nestling Black Harriers and the Transformer Density Index (Figure 18), indicating that those individuals that had the highest levels of Σ PCB in their blood were the ones that had the greater kVA-rating per km² within their territory. Electric transformers are generally considered as a potential source of PCB contamination, but this is, to the best of our knowledge, the first time that such an association is found in a wild animal. Remarkably, no association was found between Σ PCB levels and the protected area status, indicating that such areas do not reduce the risk of Black Harriers for becoming contaminated by PCBs. Even more worrying, the highest Σ PCB levels (10.0 to 13.7 ng/ml) were found for five nestlings from the West Coast National Park and the Jakkasfontein Private Nature Reserve and Koeberg Nature Reserve. All are located within the vicinity of the Koeberg nuclear power station, the only one of its type in Africa. Our Transformer Density Index, which combines transformer density and kVA, could be a useful tool to evaluate the relevance of this Σ PCB exposure route in terrestrial wildlife elsewhere.

Regarding Σ DDT (p,p'-DDT + p,p'-DDE) levels, we found that adult males had significantly higher levels than nestlings, while adult females had intermediate levels. These results were better understood when looking at differences in the variation of p,p'-DDT and p,p'-DDE concentrations, which we develop below. The levels of p,p'-DDE in nestlings were

significantly lower than those of adults, with adult males having greater levels than adult females. Although the use of DDT as an agricultural pesticide has been banned in South Africa since 1983, it remains today an important chemical in the country's fight against malaria. In the north-eastern part of the country, indoor spraying is currently used as part of the Integrated Vector Control Management Programme, within the malaria control program (Quinn et al. 2011; Ministry of Water and Environmental Affairs 2011). DDT's main metabolite, p,p'-DDE, is known to persist in the environment for many years and its toxicity remains a real threat for wildlife (Hoffman et al. 2003). In this context, the presence of p,p'-DDE in Black Harrier's blood may reflect a former use of the DDT within the species' breeding range, such as its use in agricultural lands, i.e. in crops and vineyards with evidences that stockpiles of DDT continued to be used illegally in agricultural applications into the 1990s despite of its ban (Wells & Leonard 2006), or in wetlands to control mosquitoes. While no association between Σ DDT concentrations and agricultural cover was found (such as for Σ PCB), the protected area status do not reduce the risk of Black Harriers for becoming contaminated by DDT. Our results also showed a significant increase in p,p'-DDE concentrations in Black Harrier's blood with an increasing proportion of wetland cover within the breeding territory, but no relation was found for p,p'-DDT or the Σ PCB. This suggests that p,p'-DDE concentrations reflect former DDT use for mosquito control in wetlands outside malaria-risk areas, although confirmation would be required. This contamination may also be due to the volatilization and condensation properties of POPs. Indeed, it has been suggested that POPs could volatilize from warm source/usage areas and migrate to colder regions via long range atmospheric transport, where they would later on condensate due to "cold-condensation" (Meijer et al. 2003; Ryan et al. 2012). It has also been shown that the relative concentration of high volatile POPs would increase towards colder regions (Meijer et al. 2003; Roscales et al. 2016). In this sense, the cooler temperatures occurring during the winter months in south-western South Africa, from May-June to September-October, coincident with the Black Harrier breeding season (see Chapter 1), could have contributed to their contamination. What our results do confirm is the key role played by wetlands as source of contamination by OCs in south-western South Africa and the importance of taking these areas into consideration when assessing OC exposure and implementing conservation measures, something that has also been highlighted by

Ryan et al. (2012). DDT may therefore, still be being sprayed against mosquitoes outside the malaria-risk areas despite its strict prohibition.

Unexpectedly, we found significantly higher p,p'-DDT levels in nestlings than in adult females, with adult males having intermediate levels. There are several potential explanations for this pattern. First, our study sites are located about 2,000 km away from areas where the DDT is currently sprayed for malaria control, so a direct contamination from these areas is unlikely. Nestling Black Harriers may therefore become contaminated at the breeding ground, suggesting a recent acquisition of the p,p'-DDT reflecting a current and illegal use of this pesticide, or one of its substitutes, in the region. For example, dicofol is a pesticide registered for use in South Africa and currently sprayed for the control of mites on crops and orchards, and is known to often contain p,p'-DDT (Clark 1990; Quinn et al. 2011), and to negatively impact raptor species (Clark et al. 1990; Schwarzbach et al. 1991). In this case, the legal use of dicofol may be responsible for the concentrations of p,p'-DDT found in nestlings (1.57 ± 1.88 mg/ml on average), acquired via contaminated prey in their diet.

Several studies have shown a tight link between OC concentrations and a species' diet composition, especially for species located at the top of the food chain. Fish-eating and bird-eating species are particularly vulnerable to the accumulation of OCs, and several studies have linked higher levels of PCBs and p,p'-DDE to species consuming proportionally more birds (e.g. van Drooge et al. 2008). In our study, we show that Σ DDT concentration in blood significantly increased with the percentage of bird biomass consumed by Black Harrier individuals, but this association was significant only with p,p'-DDE levels, not p,p'-DDT. Additionally, considering variation within the nestlings, we found that p,p'-DDE concentration in blood significantly increased with nestling age, confirming a bio-accumulation of this metabolite in nestling tissues over time. These results may be explained by three possible (and non-exclusive) scenarios. 1) A prior exposure of adults to the contaminant during the non-breeding season. GPS-tagged adults revealed a migration eastwards to Lesotho, the Eastern Cape and Kwazulu Natal Provinces (blog: <http://blackharrierspace.blogspot.co.za/2015/01/the-season-for-migration-east.html>). Even if these areas are geographically closer to the areas under malaria control, none of the tagged Black Harriers reached these zones, suggesting that the contamination by p,p'-DDT

may come from exposure outside malaria regions, either from illegal use, from the legal use of the pesticide dicofol, or from an atmospheric transport of OCs. 2) p,p'-DDE concentrations may reflect contamination of bird prey in other areas, in accordance with our results showing the increase of p,p'-DDE levels with higher proportion of bird biomass in an individual's diet. Indeed, Common Quails *Coturnix coturnix* represent 25.2 % of the consumed bird species by Black Harriers (see Chapter 2), and are known to migrate to areas where the DDT is still widely sprayed (e.g. Namibia, Zambia: Taylor 2005). Remaining p,p'-DDT concentrations following DDT/DDE degradation ratio (Hoffman et al. 2003) could remain present in Black Harriers' ingested prey, but in such small quantities that the relationship between diet and contamination is not significant. 3) The higher concentrations of p,p'-DDT (and Σ PCB) found in the blood of nestling Black Harriers may partly reflect a prior exposure of adult females during the non-breeding season, that would be transferred from the mother to the eggs (Drouillard & Norstrom 2001; Arenal et al. 2004; Bourgeon et al. 2013).

Maternal transfer occurs when OCs stored in lipids are mobilized and transferred to the egg and the embryo, with concentrations that can vary substantially depending on the contaminants (Hoffman et al. 2003; Arenal et al. 2004; Bustnes et al. 2008; Bourgeon et al. 2013). In our study, the Σ PCB and p,p'-DDT concentrations of adult females were always lower than those for adult males and significantly lower than those in nestlings. This is in line with findings in other bird species where contaminants are transferred to the eggs, supporting this hypothesis (Bustnes et al. 2008). In this case, adult females could excrete Σ PCB and p,p'-DDT (p,p'-DDE in much lower quantities) accumulated during the non-breeding season, at egg formation. By contrast, adult males would be continuously bio-accumulating these OCs until they biologically degrade in their bodies (Schnellmann et al. 1985; Hoffman et al. 2003). The differences in concentrations between the p,p'-DDT and p,p'-DDE between adults and nestlings may be explained by the degradation time needed to metabolize the p,p'-DDT in p,p'-DDE (Wedemeyer 1968; Hoffman et al. 2003): adults are known to have higher metabolic rates than nestlings, hence a faster capacity to degrade the accumulated p,p'-DDT into p,p'-DDE, than nestlings (Hoffman et al. 2003). This could explain why adults have higher levels of p,p'-DDE relative to nestlings, and nestlings higher levels of p,p'-DDT compared to adults. We were not able to confirm the latter hypothesis through an

analysis of egg-shell thinning, because eggshells from hatched Black Harrier nestlings are rarely found at the nests (Simmons 2000; MSGH *personal observations*).

Finally, relatively few studies have looked at the effects of the OC concentrations on the physical and physiological condition of wild raptors, which makes comparisons challenging (Rivera-Rodríguez & Rodríguez-Estrella 2011; Ortiz-Santaliestra et al. 2015). In our study, no association was found between Σ PCB and Σ DDT concentrations and the commonly used body condition index of mass corrected for age and size. This suggests that OC concentrations were unlikely explained by fat mobilization due to poorer nutritional status. On the other hand, our results showed evidences of sub-lethal effects on indicators of physiological condition: the number of white blood cells (WBC ratio) tended to increase with higher p,p'-DDT levels, and H:L ratio increased with higher Σ PCB levels in both nestlings and adults. The former may be indicative of a compensatory response of the immune system in DDT-contaminated birds; and the latter suggest an increase of the physiological stress and a reduction of the immunity for PCB-contaminated birds. A decrease of the number of lymphocytes compared to the number of heterophiles may indicate an immuno-suppressive action of PCBs, as found in other studies (Bustnes et al. 2004). Similar results were also found by Ortiz-Santaliestra et al. (2015) in a study on Bonelli's Eagle *Aquila fasciata*: adults and nestlings contaminated by PCBs, with levels similar to those of Black Harriers, exhibited a reduction of dietary antioxidant (i.e. vitamins and circulating carotenoids), and a reduced concentration of alkaline phosphatase (ALP), an indicator of osteoblastic activity. Furthermore, the levels of PCBs and p,p'-DDE found in Golden Eagles *Aquila chrysaetos*, Northern Goshawks *Accipiter gentilis* and White-Tailed Eagles *Haliaeetus albicilla* by Sonne et al. (2012) were similar to those of Black Harriers, and were also found to affect several blood clinical-chemical parameters and to be of concern for endocrine disruption and thyroid hormones.

CONCLUSIONS

This study contributes new knowledge on the exposure and transference of organochlorine compounds to wildlife in South Africa, and on how these contaminants may be affecting

top-predators, especially raptors. Using as a study model the endangered and endemic Black Harrier, our results indicate the presence of Σ PCB, p,p'-DDT and p,p'-DDE in a large proportion of the monitored population, indicating current extensive sources of contamination. Furthermore, our results linking contaminant levels and environmental variables revealed the potential sources of contamination, specifically electrical transformers for PCB exposure, and farmland birds and wetlands for p,p'-DDE exposure. Our result also showed an intra-specific relation between diet composition and OC contamination, which is usually described at the inter-specific level, and indicates that individual variation in foraging activity and prey preferences modulate the risk of exposure to these contaminants. Finally, although OCs were detected at relatively low concentrations, we could link these levels with indicators of physiological condition, suggesting that current levels of contaminant exposure represent a risk. In this context, our results on Black Harriers are relevant for other sympatric predators, especially bird-eating raptors, breeding in the same region that may equally be affected by OC contaminants. We encourage more studies on this topic in Africa where little is known on the impacts of the OC exposure on wildlife and where high light levels and temperatures are assumed to break down OCs more rapidly than in temperate areas (Hoffman et al. 2003). Our results are important to raise awareness about the potential sources of contamination and to implement future efficient conservation measures for threatened species.

Appendix D. Determining minimum samples required to estimate Black Harrier diet composition through bootstrapping analyses

When trying to quantify diet, it is important to evaluate whether the available sample size, in terms of identified prey, is sufficient to obtain an accurate estimate, and what is the threshold to determine whether to accept or reject data available. To estimate the minimum number of identified prey needed to get an unbiased estimate of Black Harrier diet composition, we conducted a series of bootstrap analyses on real diet data from a set of nests with large sample sizes. As described by Tirasin & Jorgensen (1999), bootstrapping analyses consists of drawing a large number of independent random bootstrap samples from a data set (where data is drawn by replacement), and comparing results between the original and the bootstrap samples.

Bootstrap analyses were conducted using data from 10 Black Harrier nests during the 2014 breeding season, for which we had at least 50 identified prey items. These included small mammals, birds or reptiles, with a mean of 100 prey per nest, ranging 52-156 prey. As the contribution of reptiles to diet is small, bootstrap analyses were conducted for small mammal and bird occurrence only. In other words, for small mammal occurrence, our variable had a value of 1 if the identified prey was a small mammal, and a value of 0 if it was either a bird or a reptile; sample size was thus equal to all identified prey. For bird occurrence, the variable had a value of 1 if the identified prey was a bird, and a value of 0 if it was either a small mammal or a reptile.

For each nest, we created bootstrap samples of different sizes (from 1 to the maximum sample size available for that particular nest), and calculated prey occurrence using 1,000 iterations for each sample size. For each sample size, we obtained a mean \pm SE value of each variable considered (small mammal occurrence and bird occurrence). We compare the mean (and SE) of each bootstrap sample and the mean of the full sample in relation to the sample size of each bootstrap sample.

Analyses were undertaken with R 3.2.3 (the R Foundation for statistical computing, 2015), using the package “Boot”.

Results show that estimated proportion of small mammals in the diet was very different from the original values only when the size of the bootstrap sample was less than 10 (Figure D1). Estimated proportion of birds deviated more from real values, but average values also converged from a sample size of 10 (Figure D2). Overall, the standard error (precision) of the estimated proportions decreased with increasing size of the bootstrap sample (Figure D3), but values also levelled off with sample sizes greater than 10.

This suggested that 10 identified prey is the minimum number needed to obtain an unbiased estimate of the diet composition for Black Harriers.

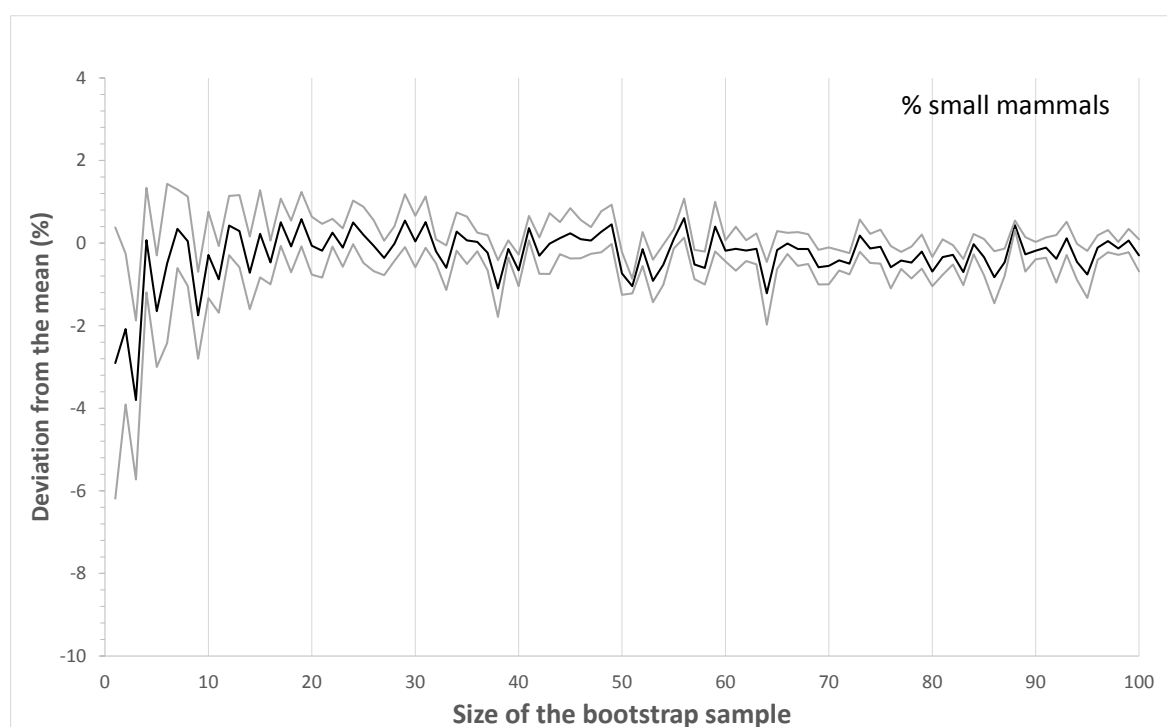


Figure D1. Mean (\pm SE) deviation (in %) between estimated and real occurrence of small mammals in the diet in relation to the size of the bootstrap sample for the 10 study nests.

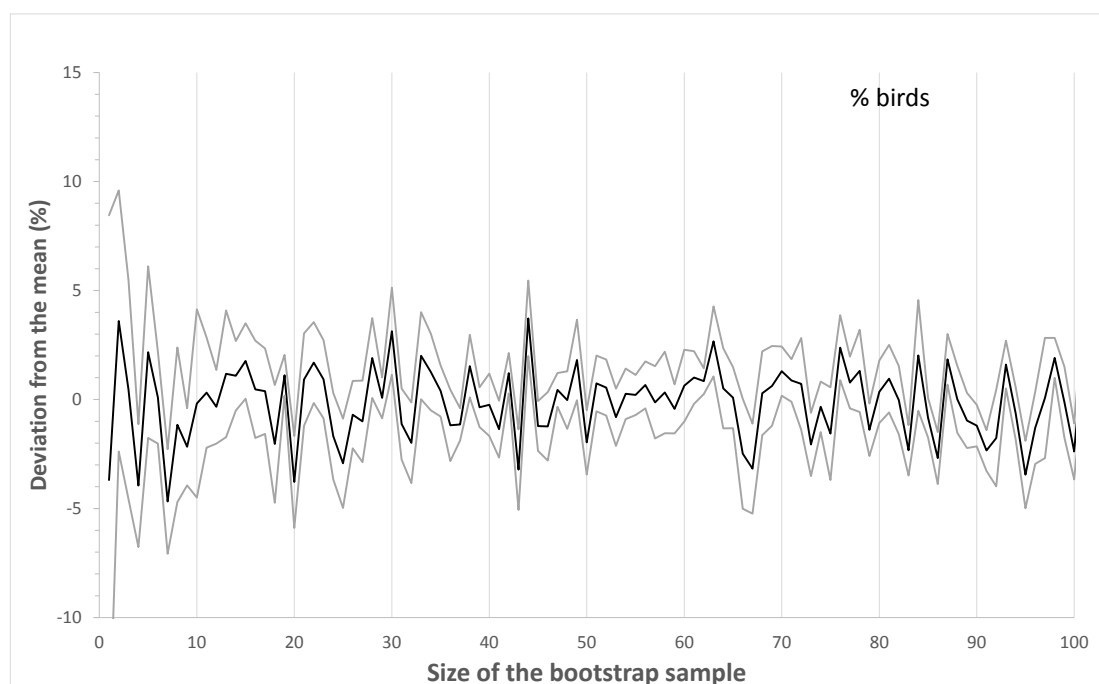


Figure D2. Mean (\pm SE) deviation (in %) between estimated and real occurrence of birds in the diet in relation to the size of the bootstrap sample for the 10 study nests.

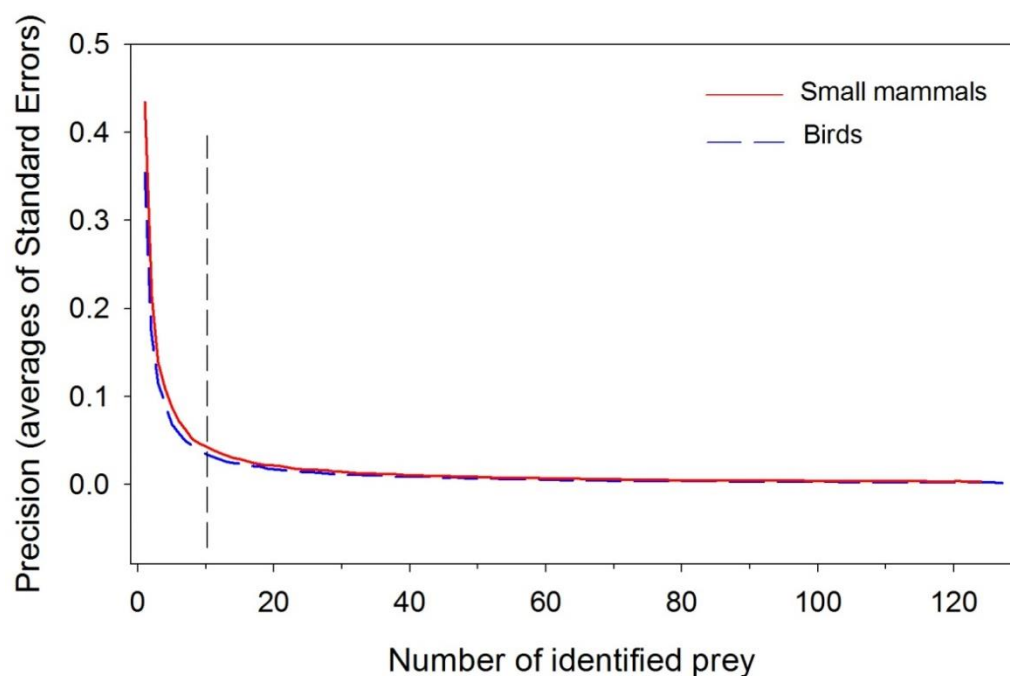


Figure D3. Average values of the Standard Errors of the estimated proportion of small mammals (red solid line) and birds (blue dashed line) in Black Harrier diet for the 10 sampled nests according to the size of the bootstrap samples. Vertical black dashed line represents the minimum number to obtain an unbiased estimate of the diet composition ($n=10$).

Appendix E. To capture adult Black Harrier breeders, a Dho Gaza net and a stuffed Spotted Eagle Owl were used (left picture). Both were set at a distance of 20-30 m from the nest to simulate a predator intrusion and elicit attacks from the adults defending the nestlings. When doing so, nestlings were a minimum of 15 days old. After being captured, adults were carefully taken out of the net; here MSGH and BA with a female Black Harrier (right picture) at the West Coast National Park. After conducting the necessary biometrical measurements and blood sampling, adults were released at the same place of capture.



Appendix F. Percentage of recovery (accuracy) and RSD (precision) of plasma samples spiked with three different concentrations of organochlorine compounds.

Compound	2.5 ng/ml (n=5)		5 ng/ml (n=5)		10 ng/ml (n=5)	
	Mean recovery	RSD	Mean recovery	RSD	Mean recovery	RSD
HCB	127	17	111	10	100	10
α -HCH	98	11	98	9	98	7
β -HCH	102	9	105	4	105	4
γ -HCH	115	12	111	7	109	6
δ -HCH	136	15	123	3	120	8
ϵ -HCH	115	12	114	4	113	3
Heptachlor	148	38	141	10	129	12
α -Heptachlor epoxide	NR	-	NR	-	11	5
β -Heptachlor epoxide	38	53	86	9	84	6
Aldrin	87	8	90	5	91	5
Isodrin	62	7	70	5	72	4
Dieldrin	NR	NR	NR	NR	NR	NR
Endrin	NR	NR	NR	NR	NR	NR
<i>trans</i> -Chlordane	110	6	111	3	110	4
<i>cis</i> -Chlordane	105	8	109	2	110	4
β -Endosulfan	NR	-	NR	-	NR	-
Mirex	111	6	115	2	110	5
<i>o,p'</i> -DDE	34	19	49	1	52	1
<i>p,p'</i> -DDE	101	7	106	2	107	2
<i>o,p'</i> -DDD	105	7	115	2	113	4
<i>p,p'</i> -DDD	112	7	117	2	113	4
<i>o,p'</i> -DDT	86	9	91	2	90	4
<i>p,p'</i> -DDT	136	14	134	2	125	8
Methoxychlor	NR	-	NR	-	NR	-
PCB 28	118	12	113	9	107	4
PCB 52	115	11	116	8	110	6
PCB 101	108	9	113	3	110	7
PCB 138	107	8	113	2	109	5
PCB 153	110	9	112	2	110	6
PCB 180	106	6	112	2	108	5

NR: Not recovered. Recovery <5%.

Chapter 5

Pollutants and diet influence carotenoid levels and integument coloration in nestlings of an endangered raptor



While awaiting for the examination of this thesis, a modified form of this chapter has been published in *Science of the Total Environment*: *García-Heras M.-S., Arroyo B. Simmons R.E, Camarero P.R., Mateo R., Mougeot F. 2017c. Pollutants and diet influence carotenoids levels and integument coloration in nestlings of an endangered raptor. Science of the Total Environment, 603-604: 299-307. Online: <http://dx.doi.org/10.1016/j.scitotenv.2017.06.048>.*

ABSTRACT

Carotenoid-based traits or ornaments, such as yellow-red integuments (feathers, beaks, legs or eye-rings) displayed by birds, play key roles in social communication by reliably advertising an individual's quality or health. In some species, these traits are displayed not only by adults but also by nestlings, and function in parent-offspring communication or sibling competition by advertising an individual's physical or physiological condition. Pollutants such as organochlorine compounds (OCs) could have disruptive effects on the coloration of these traits, thereby interfering with communication processes. Such effects have been reported in adult birds, but are still largely unknown for nestlings. Here we investigated associations between polychlorinated biphenyl (PCB) and dichlorodiphenyltrichloroethane (DDT) blood-levels, circulating carotenoid levels and the yellow-orange coloration of the cere and tarsi of wild Black Harrier *Circus maurus* nestlings, a scarce raptor endemic to southern Africa. As carotenoid pigments must be acquired through the diet, we also tested for an effect of dietary composition. The orangeness-purity of cere and tarsi coloration positively correlated with circulating carotenoid levels, and increased with both nestling age and the proportion of birds consumed in the diet. Circulating carotenoid levels and the orangeness-purity of colored integuments were unrelated to blood PCB levels, although the brightness of integuments (i.e. lack of pigmentation) increased with PCB levels. Nestlings with more DDT had lower levels of circulating carotenoids and reduced carotenoid-based coloration (i.e. higher hue and lower saturation, reflecting a yellow rather than orange and less intense color, respectively). Together, our results are consistent with the hypothesis that OC contaminants, in particular DDT, may disrupt carotenoid-based signaling in exposed nestlings. A graphical abstract representing the main results of this chapter is given in the Figure 21 below.

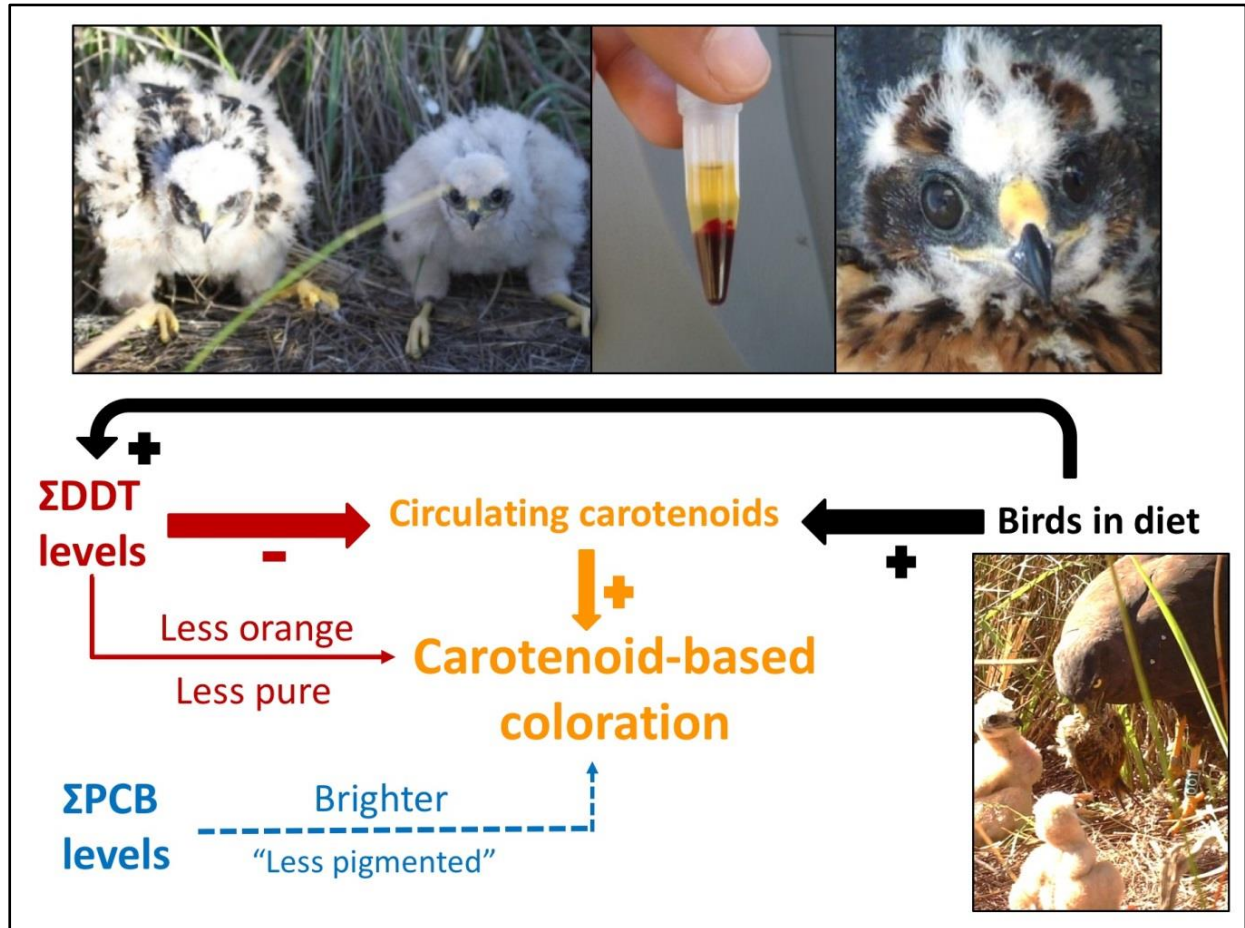


Figure 21. Graphical abstract representing the main results of Chapter 5.

INTRODUCTION

Carotenoids are pigments responsible for the red, orange and yellow coloration of integuments such as exposed skin, eye, cere, tarsus and plumages displayed by many avian species (Brush 1990; Badyaev & Hill 2000; Hill & McGraw 2006). These carotenoid-based traits or ornaments play key roles in bird communication and in social interactions (Bortolotti et al. 2000; Constantini et al. 2007; Sternalsky et al. 2009) and are displayed by both adults and nestlings, sometimes from a very young age (Bortolotti et al. 2003; Sternalsky et al. 2009, 2011, 2012). In adults, colored traits are commonly used as an honest signal of individual quality such as a superior physical condition, foraging efficiency or ability to resist parasites (e.g. Hill & McGraw 2006; Mougeot et al. 2007a). In the context of competition or mate choice, more colorful individuals are often dominant or more attractive (e.g. Goodwin 1984; Webster et al. 2008; Lindsay et al. 2011). In nestlings, however, these colored displays have other functions, and likely play a role in parent-offspring communication, i.e. for parents to assess nestling condition and needs and so adjust their feeding and caring efforts (Lyon et al. 1994), or sibling competition, i.e. for sibs to assess each-other's competitive abilities and dominance (Biard et al. 2006; Sternalski et al. 2011, 2012). Understanding the factors that influence variation in the coloration of carotenoid-based traits is particularly relevant for understanding the functions and biological importance of these traits. In that respect, little is known about the potential effects that contaminants may have on the expression of carotenoid-based traits, and the broader implications for social communication.

Vertebrates cannot synthesize carotenoid pigments *de novo* but must ingest them; thus carotenoids may be a diet-limited resource (Goodwin 1984). The total amount of carotenoid pigments available for an individual will depend on the quantity and the quality, in terms of carotenoid content, of the ingested food (Negro et al. 2002; Eeva et al. 2009). For predators, small mammal prey, for instance, are energy-rich but carotenoid-poor, while other prey such as birds, reptiles or insects have a greater carotenoid content (Goodwin 1984). Dietary composition may, therefore, be important when studying variation in circulating carotenoid levels and the expression of carotenoid-based traits (e.g. Sternalsky et al. 2009), as well as interpreting the effect of persistent organic pollutants in birds (Mañosa

et al. 2003). Carotenoids serve important health-related physiological functions: they can act as antioxidants and be used to limit oxidative damage (Pérez-Rodríguez 2009). They can also boost the immune system through immuno-stimulation and immune-regulation functions (Faivre et al. 2003; Blount et al. 2003). Carotenoid-based traits have been proposed to reliably signal an individual's healthiness, or for instance the ability to deal with parasites (e.g. Mougeot et al. 2007b). In this context, only healthy individuals could afford to deposit carotenoids to increase their ornamental coloration, rather than using them to limit oxidative damage or boost their immune defenses (Lozano 1994; Møller & Mousseau 2001; Mougeot et al. 2009; Biard et al. 2010; Pérez-Rodríguez et al. 2013). The carotenoid-based coloration may also vary with individual characteristics, including nestling age, sex (Sternalski et al. 2009, 2011, 2012), hatching order (i.e. rank within the brood) or condition (Senar et al. 2003; Sternalsky et al. 2009, 2012). It seems, therefore, important to consider these variables, as they may help explain variations in physiological aspects that may ultimately influence future reproductive outcomes of breeding birds (Eeva et al. 2012; Sassani et al. 2016).

Carotenoid-based coloration and circulating carotenoids have been shown to be affected by both non-organic contaminants such as heavy metals (Camplani et al. 1999; Møller & Mousseau 2001; Eeva et al. 2008, 2009; Giraudeau et al. 2015; Vallverdú-Coll et al. 2015, 2016a) and organic contaminants such as persistent organic pollutants (POPs) (McCarty & Secord 2000; Bortolotti et al. 2003; López-Antia et al. 2013; Blévin et al. 2014; López-Antia et al. 2015a, 2015b) or fuel oil pollution (Pérez et al. 2010). Pollutants could thereby potentially interfere with communication processes that rely on carotenoid-colored traits (see Marasco & Costantini 2016). For example, the carotenoid-based coloration of eye-rings, gapes and tongues of adult female Kittiwakes *Rissa tridactyla* decreased with increasing levels of POPs in the blood (Blévin et al. 2014). By contrast, no association was found between POPs contamination and the carotenoid-based coloration of integuments of breeding Great Black-Backed Gulls *Larus marinus* (Bustnes et al. 2007). Several studies have related exposure to pollutants to both circulating carotenoids and carotenoid-based coloration (López-Antia et al. 2013, 2015a, 2015b; Vallverdú-Coll et al. 2015, 2016a; García-de Blas et al. 2016), but few of them have been performed with birds in the wild (Bortolotti et al. 2003; Eeva et al. 2008; Vallverdú-Coll et al. 2016b). In addition, most studies have

been conducted on adult birds, but relatively few have been undertaken with nestlings and the knowledge and understanding of how these contaminants affect them is particularly lacking.

The main goal of the present study was to investigate whether OCs affect the circulating carotenoid levels and the carotenoid-based coloration of integuments (yellow-orange cere and tarsi) developed by Black Harrier *Circus maurus* nestlings. Unlike feathers, the carotenoid-based coloration of fleshy integuments is dynamic, with the potential for rapid change (Velando et al. 2006; Pérez-Rodríguez 2008). Therefore, changes in coloration of integuments such as cere and tarsi and in circulating carotenoid levels may reflect a more recent intake of carotenoids and recent changes in individual condition (Faivre et al. 2003; López et al. 2011).

The Black Harrier is a scarce medium-sized raptor, endemic to southern Africa, and classed as Endangered in South Africa, Namibia and Lesotho (Simmons et al. 2015; Taylor 2015). This ground-nesting bird of prey breeds in indigenous vegetation of south western South Africa, preferentially along the coast within the Fynbos biome, and also inland within the Karoo biome (Curtis et al. 2004; Curtis 2005; Chapter 1). Like most raptors, Black Harriers display carotenoid-based coloration from a young age, i.e. a few days after birth. Black Harrier broods include generally 2 to 4 nestlings (Simmons 2000; Simmons et al. 2005) and competition for food can be intense in harsh conditions, sometimes leading to chick mortality (Simmons 2000; MSGH, *personal observations*). A recent study revealed the presence of OCs in both adults and nestlings (see Chapter 4). PCBs (PCBs 52, 101, 138, 153, 180) and DDTs (p,p'-DDT and its metabolite p,p'-DDE) were detected in the blood plasma of 82% and 81% of nestlings, respectively. Detected concentrations were high enough to induce physiological effects, such as increased heterophil to lymphocyte ratio in PCB-exposed individuals and increased white blood cell counts in DDT-exposed individuals, but had no effect on nestling's body condition (see Chapter 4). Black Harriers prey mostly on small mammals, but also on birds such as Common Quails (*Coturnix coturnix*) as alternative prey (see Chapters 2 & 3). Birds are generally known to bio-accumulate more OCs than small mammals (Fossi et al. 1995; Mañosa et al. 2003; van Drooge et al. 2008), and in Chapter 4 we found a positive association between blood-p,p'-DDE levels and the proportion of bird

biomass in Black Harriers diet. In this context, diet may affect carotenoid availability directly because small mammals are carotenoid-poor prey compared with birds, as well as indirectly because, in this region, individuals eating more birds are also more exposed to DDTs (see Chapter 4)

We first tested whether carotenoid levels and carotenoid-based coloration varied with nestling age, sex, rank, physical condition and dietary composition. We expected well-fed nestlings in better condition (i.e. mass corrected for age) to be more colored, and individuals eating more birds to be less carotenoid-limited and more colored. Second, we tested whether circulating carotenoid and coloration varied with DDT and PCB levels, and expected exposed nestlings to display less colored integuments, because they may allocate available carotenoids towards detoxification to counter adverse physiological effects of OCs.

MATERIALS AND METHODS

Study Sites and data collection

Fieldwork was conducted in South Africa during the 2012, 2013 and 2014 Black Harrier breeding seasons (July to December). Study nests were located along the coast of the Western Cape Province (33.700°S, 18.450°E; 33.133°S, 18.083°E) and inland in the Northern Cape Province in the Nieuwoudtville area (31.316° S, 19.083° E). Breeding sites were in National Parks (South African National Parks – SANParks properties), Provincial Protected Reserves (Cape Nature), or on private land (see Chapter 1).

Nestlings were measured and sampled during nest visits when 15-39 days old (n= 118, 63 females and 55 males; see Chapter 1 and Appendix A for more details). Each was individually marked with a metal and a color ring, with a unique alpha-numeric code. The rank of each nestling within the brood (i.e. hatching order, rank 1 being the first hatched chick) was also determined from nestling age, itself determined from biometrics (Appendix A). We measured tarsus length to the nearest 0.1 mm using an electronic caliper, and body mass to the nearest 5-10 g using a Pesola balance, and subsequently calculated a body condition index (i.e. physical condition) using the residuals from the relation between the

body mass and the age of nestlings, calculated for each sex separately (see Chapter 4 or Appendix A).

We collected a blood sample from the brachial vein (0.7-1 ml) using a heparinized syringe. The blood was stored in a heparinized Eppendorf vial and kept in a polystyrene cool box previously filled with ice blocks. Within 30-40 min after collection, the samples were centrifuged for 15 min with a mini portable centrifuge (VWR, Radnor, Pennsylvania) to separate the plasma from the red cells. Both set of samples were immediately placed in a portable freezer, and frozen at -80°C on arrival at the lab from the field (< 3 hours after collection) until analysis. Plasma samples were used to identify and quantify the concentrations of OCs and circulating carotenoids. Red blood cell samples were used to determine the sex of nestlings by molecular procedures using the primers 0057F and 002R (Round et al. 2007) and protocols described in Chapter 4. We obtained blood samples from a total of 101 nestlings from 44 nests. Twenty-three nestlings were blood sampled twice: at 15-20 days old, and again at 30-39 days old. Fieldwork protocols were approved by the University of Cape Town's science Faculty Animal Ethics Committee (Permit number: A1/2014/2013/V21/GC).

Dietary composition

Diet information was available for a total of 29 nests and 63 nestlings. We assessed dietary composition at each nest either by analyzing bones, scales, feathers or hairs within pellets collected at the nest (n= 17 nests), by analyzing video and picture footage from cameras set at nests (n= 2 nests), or by using a combination of both techniques (n= 10 nests). Estimates of diet from pellets and cameras correlated with each other when the proportion of unidentified prey in cameras was low (see Appendix B). Identified prey were categorized as small mammal, bird or reptile, and the percentage biomass contributed by each prey category was estimated by allocating an average weight to each category (see Chapter 2 for more details). The contribution of reptiles to consumed biomass was <5%, and the proportion of bird biomass was thus strongly negatively related to the proportion of small mammal biomass (Pearson correlation: $r = -0.98$, $p < 0.0001$, $n = 29$). Therefore, variations in diet composition are reflected by variations in the proportion of bird biomass, and we only

used this variable in analyses, as an indicator of dietary composition. An average (\pm SE) of 55.4 ± 0.21 prey items (min-max: 10-164) were identified for each nest. Ten prey was determined to be sufficient for an accurate estimation of the proportion of birds or small mammals in the diet using bootstrapping analyses (see Appendix D).

Plasma concentrations of organochlorine compounds and carotenoids

OCs were identified and quantified using the protocol described in Chapter 4. In short, we looked for PCB and DDT components present in the Pesticide-Mix 13 (Dr. Ehrenstorfer standard). Plasma samples were extracted and cleaned up following the methods described and validated by Mateo et al. (2012). These are based on the sulfuric acid clean-ups using the n-hexane extraction procedure, and organochlorine concentrations were measured by gas chromatography coupled to an electron capture detector (GC-ECD) equipped with a column HP-5 30 m, 0.32 mm, 0.25 μ m, both from Agilent Technologies. OC levels (expressed in ng/ml) were determined for 90 nestlings comprising 50 females and 40 males, each nestling sampled only once.

Carotenoids were extracted from the plasma following a procedure validated by García-de Blas et al. (2013), and identified and quantified using high performance liquid chromatography (HPLC) coupled with a photodiode detector. Briefly, 50 μ l of plasma were put into a polypropylene microtube, in which 200 μ l of distilled water and 150 μ l of ethanol were added. The headspace tube was filled with nitrogen to avoid carotenoid oxidation, vortexed for 5 min and vibrated with ultra-sound (sonicated) for 1 min. The mixture was then extracted three times with 1 ml of hexane using vortex mixing for 15 min each time. Hexane phases were recovered after centrifuging for 5 min at 2,500 g and at 4°C. The supernatant (i.e. hexane phase) was extracted, combined and put into a long tube kept in the dark. The supernatant was then evaporated to dryness under a stream of nitrogen. Residues were immediately re-dissolved with 100 μ l of methanol, transferred to a glass vial for HPLC analysis with an Agilent Technologies 1200 Series. The HPLC method used to separate, identify and quantify the carotenoid pigments present in the samples is described in Rodríguez-Estival et al. (2010). We identified two main carotenoids in the plasma of nestling Black Harriers, lutein and zeaxanthin (see Appendices G & H), as found in most raptor species (Hill & McGraw 2006). Based on a sub-sample of 67 analyzed plasma, we

found that lutein and zeaxanthin levels were strongly positively correlated ($r= 0.88$, $p<0.0001$, $n= 67$). To simplify the statistical analyses, and because in some chromatograms both substances co-eluted, we summed the lutein and zeaxanthin levels and used a total carotenoid concentration in all analyses. Circulating carotenoid levels (expressed in nmol/ml) were thus determined for a total 101 nestlings, 58 females and 43 males.

Carotenoid-based colouration

To measure the carotenoid-based coloration, we took digital photographs of the cere and tarsi of each nestling using a Panasonic DMC-FZ38 digital camera. All pictures were taken by the same person (MSGH) with the same camera, at a standard distance of 40 cm between the objective lens and the body parts, avoiding direct sun light. The same yellow board card and color chart were placed next to each photographed body part (i.e. cere or tarsus) to standardize color measurements. Each integument was photographed three times, and the best quality picture (one for each integument part) was selected for analysis. Photographs were analyzed using Adobe Photoshop (CS5), a method used previously to study coloration in raptors (Martínez-Padilla et al. 2013) or other birds (Mougeot et al. 2009; Vallverdú-Coll et al. 2016a). For each body part, we selected a homogeneous area with the magic wand tool, and calculated its three-color characteristics in the HSB color space: hue (H), saturation (S) and brightness (B). These measurements were repeated four times and we used average values. We did the same with the yellow standard reference to standardize the body part measurements among all photographs.

The hue reflects the perceived color of the integument (e.g. yellow, orange, red), while the saturation indicates the intensity and the purity of a color. The brightness reflects the “quantity of white” present in each color (Hill & McGraw 2006). The digital photography method was preferred to other methods, such as spectrophotometry, because of fieldwork constraints and because it is better suited to quantify the coloration of irregular surfaces such as ceres and tarsii (Montgomerie 2006). We obtained color measurements of both for a total of 115 nestlings (54 males and 61 females), with 37 of these being measured up to three times during their growth.

The values of hue, saturation and brightness of the cere and the tarsi were positively correlated (Pearson correlations: Hue: $r = 0.32$, $p < 0.0001$, $n = 115$; Saturation: $r = 0.64$, $p < 0.0001$, $n = 115$, Brightness: $r = 0.43$, $p < 0.0001$, $n = 115$). Therefore, to reduce the number of coloration variables and to account for collinearity among them, we conducted a Principal Component Analysis (PCA) on the 6 coloration variables, i.e. hue, saturation and brightness of both cere and tarsi. The first Principal Component (hereafter coloration-PC1) explained 44% of the variance, and the second Principal Component (hereafter coloration-PC2) explained a further 23% of the variance (Table 10). High values of coloration-PC1 were indicative of a lower hue value and a greater saturation, i.e. cere and tarsi that were more orange than yellow, and with a purer and more intense coloration. This was indicative of a greater carotenoid-based coloration of these integuments. High values of coloration-PC2 were indicative of a greater brightness (whiteness), thus indicating integuments that were less pigmented overall, with more reflectance from the unpigmented (i.e. white) skin (Mougeot et al. 2007a).

Table 10. Results of the Principal Component Analysis conducted on the six coloration measurements (i.e. hue, saturation and brightness of the cere and tarsi) for Black Harrier nestlings ($n = 124$). Variable loadings greater than 0.4 or lower than -0.4 are highlighted in bold.

	PC1	PC2
Cere Brightness	-0.18	0.68
Tarsus Brightness	-0.01	0.71
Cere Hue	-0.48	-0.18
Tarsus Hue	-0.42	0.04
Cere Saturation	0.53	0.08
Tarsus-Saturation	0.53	0.04
Variance explained		
Proportion	0.44	0.23
Cumulative		0.67

Statistical analyses

All statistical analyses were conducted using R 3.2.3 (the R Foundation for statistical computing, 2015).

Because sample sizes varied depending on the variables measured (i.e. not all data could be measured for all nestlings), we conducted two sets of General Linear Mixed Models (GLMMs) to investigate how coloration and circulating carotenoids varied with 1) age, sex, nestling rank, body condition index, and dietary composition (n= 93 samples from 63 nestlings from 29 nests,) and 2) age, diet, and contaminants (Σ PCB and Σ DDT, which includes p,p'-DDE and p,p'-DDT) (n= 56). We initially also considered lay date as an explanatory variable in our models, but it was correlated with dietary composition (the occurrence of birds in the diet of nestling increases as the breeding season progresses; see Chapter 3). To avoid collinearity issues, we thus only considered the % of bird biomass in our statistical models, considering this variable more biologically meaningful to explain carotenoid intake and availability. Circulating carotenoid levels were log-transformed to adjust for normality. Dependent variables were fitted to models using a normal distribution (package lme4, log function, Bates et al. 2012). Nest identity and nestling identity were included as random effects in all models to account for the non-independence of samples coming from the same nest and the repeated measures on some nestlings.

In the first set of models, we tested whether the coloration PCs and circulating carotenoid levels varied with age (from 15-39 days old, continuous variable; reflecting changes during nestling growth), sex, brood rank (from 1 to 4, continuous variable; reflecting effects of hatching asynchrony and nestling competition), body condition index (continuous variable) and dietary composition (proportion of ingested bird biomass, square root transformed to adjust better to a linear model). We also included the interaction age \times sex. Non-significant interactions and variables were removed from the initial models using a stepwise backward procedure and we present the results of the final models (Type III results).

In the second set, we investigated additional effects of OC levels (Σ PCB as the sum of all detected PCBs, and Σ DDT as the sum of all detected DDTs) on coloration and circulating carotenoids, adding these variables as explanatory variables to those that were retained in

previous models. We checked for correlations and collinearity among these explanatory variables and calculated their Variance Inflation Factors (VIF). All VIF values were below 1.4, indicating that collinearity was not an issue (Zuur et al. 2010). For a better adjustment to a linear model, Σ PCB was log-transformed. These models were performed using data from 56 individuals (each sampled once).

RESULTS

Plasma concentrations of carotenoids averaged 10.7 ± 6.5 nmol/ml (minimum-maximum: 0.7-36.7). Coloration-PC1 (i.e. hue-saturation) was positively correlated with circulating carotenoids ($\chi^2 = 38.37$, d.f.= 1, $p < 0.0001$; slope: $+1.270 \pm 0.205$; $n = 93$), indicating that higher circulating carotenoid levels were associated with a more orange and purer coloration of integuments (Figure 22a). No significant correlation was found between coloration-PC2 (i.e. brightness) and circulating carotenoids ($\chi^2 = 0.029$, d.f.= 1, $p = 0.865$; $n = 93$) (Figure 22b).

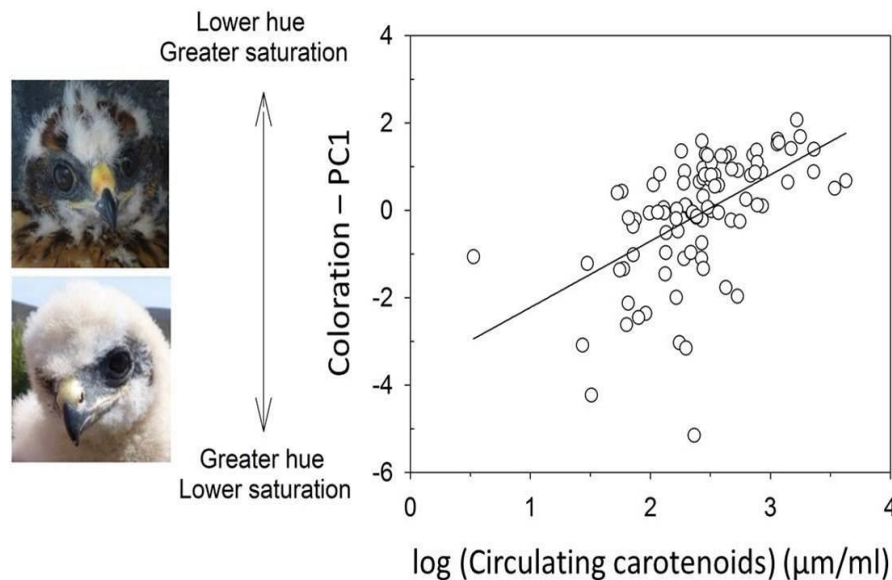


Figure 22. Relationships between circulating carotenoids and (a) the colouration-PC1 (i.e. hue-saturation), (b) the colouration-PC2 (brightness) obtained from colour measurements of the cere and tarsi of wild Black Harrier nestlings ($n = 124$). Solid black line highlights significant relationship (a).

Carotenoid-based coloration, body condition and dietary composition

Circulating carotenoids and coloration-PC1 increased with nestling age: older nestlings had more circulating carotenoids and developed a more orange and purer coloration than younger ones (Table 11, Figure 23a, d). The coloration-PC2, however, did not vary with age (Table 11). Circulating carotenoids, coloration-PC1 and PC2 did not differ between sexes, and were not associated with brood rank or body condition index (Table 11). The latter indicated that relatively heavier nestlings were not more colorful than lighter ones.

Circulating carotenoids and coloration-PC1 both increased with the percentage of bird biomass in nestlings' diet (Table 11, Figure 23b, e). Nestlings consuming more birds had more circulating carotenoids and integuments of a more orange and purer color than those of nestlings consuming more small mammals. No association was found between diet and coloration-PC2 (Table 11).

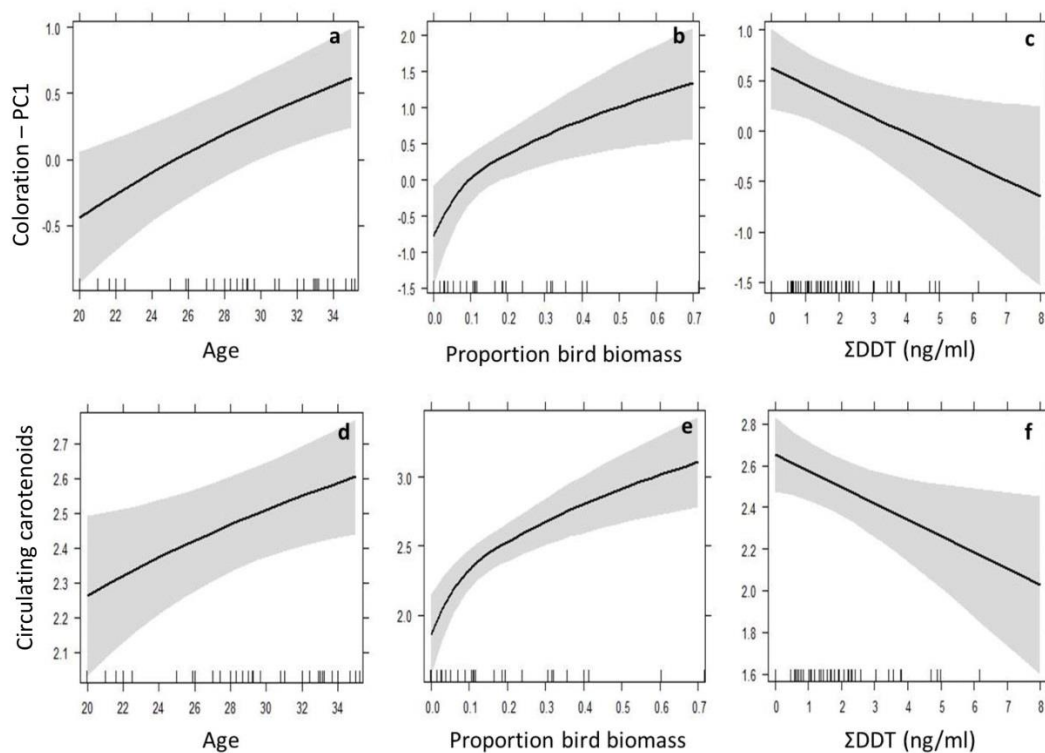


Figure 23. Results of the models (fitted curve: black lines; 95% confidence intervals in grey) testing for associations between coloration-PC1 or circulating carotenoid levels and: (a,d) nestling age, (b,e) dietary composition (% of bird biomass in diet), (c,f) ΣDDT levels. Model outputs are given in Table 12. Plots with the raw data are shown in Appendix I.

Table 11: Results of GLMMs testing for the effects of nestling age, sex, body condition index, rank and dietary composition (% of bird biomass in diet) on circulating carotenoid levels and carotenoid-based coloration (coloration-PC1: hue-saturation; coloration-PC2: brightness; see Table 10). Significant variables are highlighted in bold, and parameter estimates for those variables are also included. Models were performed using 93 data points from 63 nestlings. Nest and nestling identity were both included as random effects in models. d.f. = degrees of freedom.

Independent variables	Explanatory variables	Chi-square	d.f.	P	Estimate \pm SE
Circulating carotenoids*					Intercept: 0.821 ± 0.528
	Age*	4.123	1	0.042	0.306 ± 0.151
	% Bird in diet[§]	23.953	1	<0.0001	1.511 ± 0.309
	Sex	0.156	1	0.693	
	Body condition	0.805	1	0.369	
	Rank	0.003	1	0.959	
Coloration-PC1					Intercept: -10.547 ± 1.548
	Age*	40.831	1	< 0.0001	2.846 ± 0.445
	% Bird in diet[§]	10.643	1	0.001	2.535 ± 0.777
	Sex	1.628	1	0.202	
	Body condition	1.137	1	0.286	
	Rank	1.539	1	0.215	
Coloration-PC2					
	Age*	0.909	1	0.340	
	% Bird in diet [§]	0.961	1	0.327	
	Sex	0.425	1	0.515	
	Body condition	1.174	1	0.259	
	Rank	0.026	1	0.872	

* log transformed

[§] square-root transformed

Carotenoid-based coloration and organochlorine compounds

In sampled nestlings, Σ PCB levels averaged 3.5 ± 3.1 ng/ml (minimum-maximum: 0-13.7 ng/ml), while Σ DDTs (p,p'-DDE + p,p'-DDT) levels averaged 1.9 ± 2.0 ng/ml [minimum-maximum: 0-9.8 ng/ml]. We found a significant negative association between DDT levels and carotenoid levels, coloration-PC1 and coloration-PC2 (Table 12). Nestlings with more DDTs circulated fewer carotenoids (Figure 23f) and had yellower rather than orange, less

saturated and less bright colored traits. Coloration-PC2 was positively associated with PCB levels: nestlings exposed to PCBs displayed brighter (i.e. whiter) cere and tarsi. No significant associations were found between PCB levels and circulating carotenoids or coloration-PC1 (Table 12).

Table 12. Results of GLMMs testing for the effects of age, dietary composition (% of bird biomass in diet), and Organic Compound (Σ PCB and Σ DDT) levels on circulating carotenoids and carotenoid-based coloration (Coloration-PC1: hue-saturation; Coloration-PC2: brightness; see Table 10). Significant variables are highlighted in bold, and parameter estimates for those variables are also included. Models were performed with 56 nestlings. Nest identity was included as a random effect in models. d.f. = degrees of freedom.

Independent variables	Explanatory variables	Chi-square	d.f.	P	Estimate \pm SE
Circulating carotenoids*					Intercept: -0.184 ± 0.919
	Age*	5.966	1	0.015	0.636 ± 0.260
	% Bird in diet[§]	20.001	1	< 0.0001	1.494 ± 0.334
	ΣDDT	5.782	1	0.016	-0.079 ± 0.033
	Σ PCB*	1.306	1	0.253	
Coloration-PC1					Intercept: -7.262 ± 1.928
	Age*	12.976	1	0.0003	1.971 ± 0.547
	% Bird in diet[§]	10.227	1	0.001	2.527 ± 0.790
	ΣDDT	5.684	1	0.017	-0.159 ± 0.066
	Σ PCB*	1.839	1	0.175	
Coloration-PC2					Intercept: 0.211 ± 0.278
	ΣDDT	5.150	1	0.023	-0.180 ± 0.079
	ΣPCB*	4.385	1	0.036	0.394 ± 0.188
	Age*	0.339	1	0.560	
	% Bird in diet [§]	1.128	1	0.288	

* log transformed

[§] square-root transformed

DISCUSSION

We investigated the associations between diet, OCs, carotenoid-based coloration, and circulating carotenoid levels in nestlings of a wild raptor species, and contribute novel knowledge by revealing that both diet and OCs contamination influence the expression of carotenoid-based traits displayed by nestlings. Detected OC levels in our study were within the range or slightly higher than those found in recent studies conducted on other raptors (e.g. Sonne et al. 2012; Bustnes et al. 2013; Eulaers et al. 2014; Gómez-Ramírez et al. 2014), and for which adverse physiological effects have been reported (e.g. Ortiz-Santaliestra et al. 2015).

Variation in carotenoid levels and coloration according to age and body condition

Black Harrier nestlings circulating more carotenoids had a more orange and purer coloration of integuments. The orangeness of cere and tarsi and the circulating carotenoid levels increased with nestling age. This may reflect an increasing absorption, deposition and accumulation of circulating carotenoids throughout nestling growth (distinguishing it from carotenoids received from the egg yolk, or stored in fat or in the liver; Hill & McGraw 2006). Such accumulation of carotenoids can be allocated towards nestling coloration or for self-maintenance needs (Casagrande et al. 2007; see also below). As carotenoid pigments in animals must be acquired through the diet, their accessibility will depend on the type of food, but could also depend on the quantity of the ingested food. When prey type does not vary, nestlings that receive more food would have also acquired more carotenoids and should be more colored as well as in better physical condition (relatively heavier). Under such a scenario, carotenoid-based coloration is expected to be condition-dependent (Hill, 1990; Senar et al. 2003; Tschirren et al. 2003; Biard et al. 2006; McGraw & Hill 2006). In our study, however, no significant associations were found between carotenoid-based coloration or circulating carotenoids and a nestlings' body condition index: heavier nestlings were not more colorful, nor did they have more circulating carotenoids than lighter ones. This may arise when carotenoid intake primarily depends on the quality (carotenoid content) of the ingested food rather than its quantity. This concurs with results found in wild Montagu's Harrier *Circus pygargus* and Western Marsh Harrier *Circus aeruginosus* nestlings

(Sternalsky et al. 2009, 2012), and in Great Tits *Parus major* nestlings (Eeva et al. 2009). It may also explain why we did not find differences in carotenoid levels or coloration between male and female nestlings, or according to nestling rank (hatching order), as reported elsewhere. These differences are usually associated with differences in food quantity rather than quality among nestlings of different sex or rank (Magrath 1990; MacWhirter 1994; Bortolotti et al. 2003; Sternalsky et al. 2009, 2012).

Variation in carotenoid levels and coloration according to diet and OC levels

While the coloration of feathers reflects an earlier availability and accumulation of carotenoid pigments (during moult), the coloration of integuments such as cere and tarsi usually reflects a more recent intake or mobilization of carotenoids (López et al. 2011). As expected, we found that nestlings feeding on a diet rich in birds had higher levels of circulating carotenoids and displayed a greater carotenoid-based coloration than those feeding regularly on small mammals. Our results are in accordance with other studies showing the tight link between the expression of the carotenoid-based coloration and the type and amount of carotenoid pigments ingested by wild vertebrates (Hill et al. 2002; Negro et al. 2002; Eeva et al. 2008, 2009; Sternalski et al. 2009). Indeed, small mammals are known to be carotenoid-poor, unlike other birds, reptiles and insects (Goodwin 1984). In this context, feeding on small mammals, the Black Harrier's primary prey (Chapters 2 and 3), may be profitable for nestlings in terms of energy gain, but consuming more birds seems also to be beneficial as it increases carotenoid availability; this in turn has health-related physiological benefits (Lozano 1994). On the other hand, we also demonstrated that the greater the proportion of ingested bird biomass in the diet, the higher the levels of blood Σ DDT in nestlings and in adults (Chapter 4). This is consistent with other studies that reported a positive link between the consumption of bird prey and OC exposure (Fossi et al. 1995; Mañosa et al. 2003; van Drooge et al. 2008). Toxic substances in diet, such as Σ DDT, can impose stress that alters nutrient requirements or use (Phillips & Hidiroglou 1965). For instance, rats fed over 10 mg/kg of DDT in the diet decreased utilization of orally administrated carotene and vitamin A (Phillips 1963). Similarly, cows fed with forage containing 40 to 60 mg/kg of o,p'-, p,p'-DDT (dry weight) exhibited lower levels of

carotenoids and vitamin A in both serum and liver (Phillips & Hidioglou 1965). It may be reasonable to expect a reduction in the levels of circulating carotenoids in Black Harrier nestlings with higher levels of DDT, which in turn may lead to a reduced carotenoid-based coloration of integuments. Furthermore, several organic contaminants (e.g. PCBs and perfluoroalkoxy alkanes -PFAs) have been shown to alter various physiological processes, causing changes in endocrine (Rattner et al. 1984) and neuroendocrine pathways (Frye et al. 2011) and in oxidative balance (Whysner & Wang 2001). These changes may induce a disruption of the coloration of integuments and/or plumages (Surai 2002; Bortolotti et al. 2003; Marasco & Costantini 2016). Our results indeed show that DDT-contaminated Black Harrier nestlings had significantly lower levels of circulating carotenoids and reduced carotenoid-based coloration (yellow rather than orange and less saturated integuments). In this context, the beneficial “extra-amount” of circulating carotenoids obtained by harrier nestlings eating birds (rather than small mammals) seems to be compromised when consuming birds that simultaneously expose harriers to DDT-contamination. Future experimental studies will, however, be necessary to confirm this. Additionally, we found a negative association between DDT levels and coloration-PC2 indicating a reduced brightness of integument color. This is rather counter-intuitive, because a reduced brightness (lower PC2 values) would be indicative of a less white, more pigmented integument. Since PC2 is unrelated to circulated carotenoid levels and unrelated to PC1 by definition, this result suggests that the two characteristics of integument coloration (hue and saturation versus brightness) are affected by contaminants via different pathways (the former being more clearly linked with carotenoid availability than the latter).

To the best of our knowledge, our study is the first to report an association between blood-DDT levels, integument coloration and circulating carotenoids in a bird species. Similar results were, however, found in amphibians where DDT-congeners have been shown to negatively affect the endocrine system in the Asian Common Toad *Bufo melanostictus* and in the Reed Frog *Hyperolius argus*, resulting in changes in the skin-coloration of individuals (Noriega & Hayes 2000).

We also found that the brightness of cere and tarsi color significantly increased with blood-PCB levels. As emphasized above, it is unclear to what extent the brightness of cere

and tarsi is dependent upon carotenoid availability and allocation priorities. Higher PC2 values could be indicative of whiter, less pigmented integuments. Alternatively, variation in integument brightness could be unrelated to carotenoid availability. PCBs are known to activate the cytochrome P450 enzyme system (which acts on the cycles of oxidation-reduction and generates free radicals; Schlezinger et al. 1999), and may thereby increase the production of pro-oxidant molecules, which may in turn cause oxidative stress. PCBs may also cause a de-regulation of specific antioxidants, which in turn would also amplify the effects of oxidative stress (Marasco & Constantini 2016), and concomitantly affect the brightness of integument coloration of Black Harrier nestlings. By increasing oxidative stress, PCBs could affect carotenoid allocation priorities: carotenoid pigments have antioxidant properties and their allocation to colored integuments is usually reduced under conditions of increased oxidative stress (e.g. Mougeot et al. 2010; Pérez-Rodríguez et al. 2010; Vallverdu-Coll et al. 2015, 2016a).

In Chapter 4, we found no association between the levels of OCs (DDTs or PCBs) in nestlings' blood and their body condition. Here, we found no relationships between carotenoid-based coloration or circulating carotenoid levels and the body condition of nestlings, indicating that the reported effects of OCs, and in particular of DDT, on carotenoid-based coloration were unlikely to be mediated via effects on body condition, but would rather be mediated by physiological effects (e.g. detoxification need, increased oxidative stress or reduced immunity).

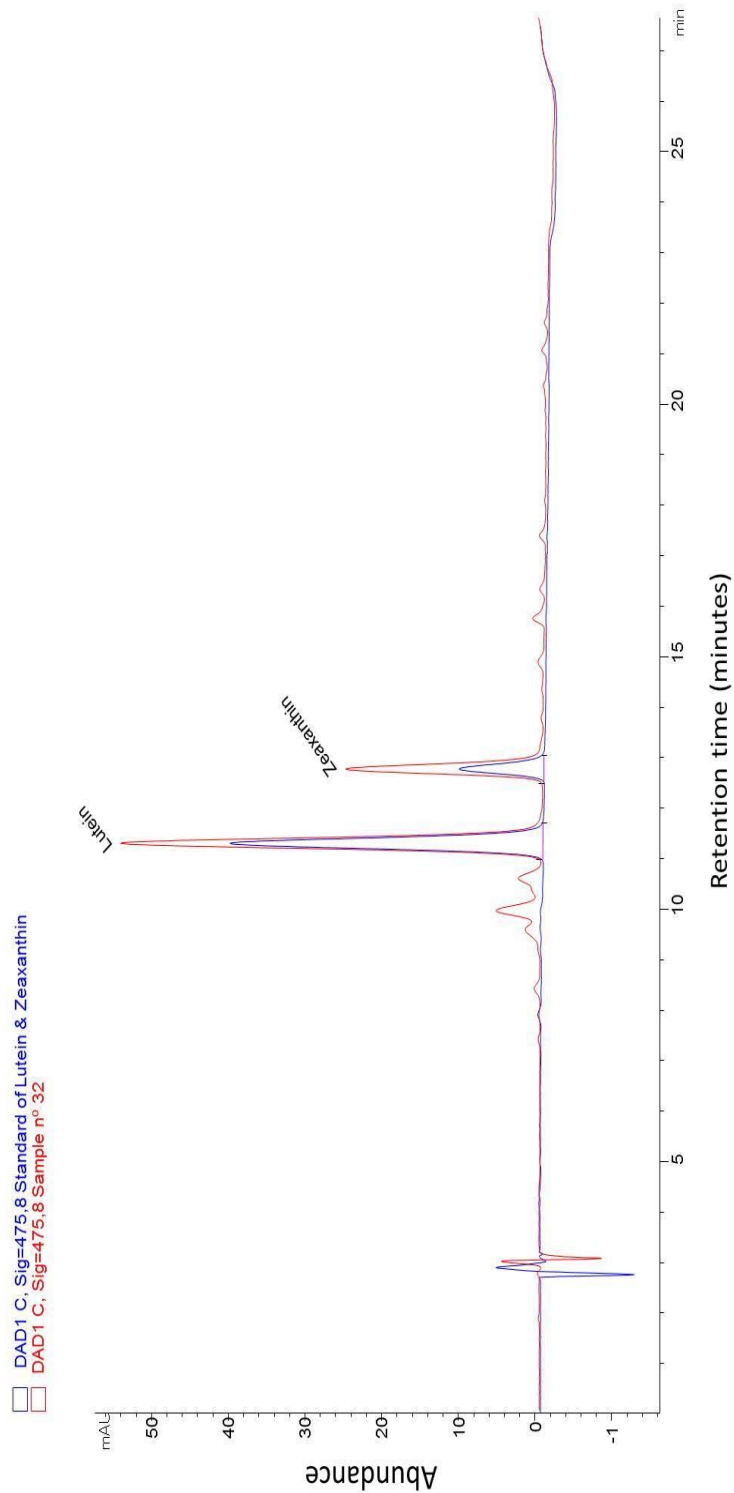
PCB- (and DDT-) exposure may also have important health implications for nestlings, which may be more vulnerable to other threats, for example more prone to parasite infection (López et al. 2011). Only a few studies have related OC exposure to indicators of health, but with inconsistent results (Bustnes et al. 2004, 2007; Rivera-Rodríguez & Rodríguez-Estrella 2011; Ortiz-Santaliestra et al. 2015). While Bustnes et al. (2004) showed an increase of the numbers of heterophils with higher OC levels in adult breeding Glaucous Gulls *Larus hyperboreus*, no association between white blood cell counts and OC levels were found in the Great Black-Backed Gulls *Larus marinus* (Bustnes et al. 2007). In adult and nestling Bonelli's Eagles *Aquila fasciata* contaminated with PCBs at similar levels to the Black Harriers in our study, Ortiz-Santaliestra et al. (2015) found a reduction of dietary

antioxidants (i.e. circulating carotenoids) and of alkaline phosphatase (indicators of osteoblastic activity). Similarly, levels of PCBs and DDTs equivalent to Black Harriers' were found to affect several blood clinical-chemical parameters in Golden Eagles *Aquila chrysaetos*, Northern Goshawks and White-Tailed Eagles *Haliaeetus albicilla* (Sonne et al. 2012; Bustnes et al. 2013; Eulaers et al. 2014). In this context, further studies should be undertaken to investigate how these OCs may physiologically compromise Black Harrier nestlings as appears to be the case in other raptors (Rivera-Rodríguez & Rodríguez-Estrella 2011; Ortiz-Santaliestra et al. 2015).

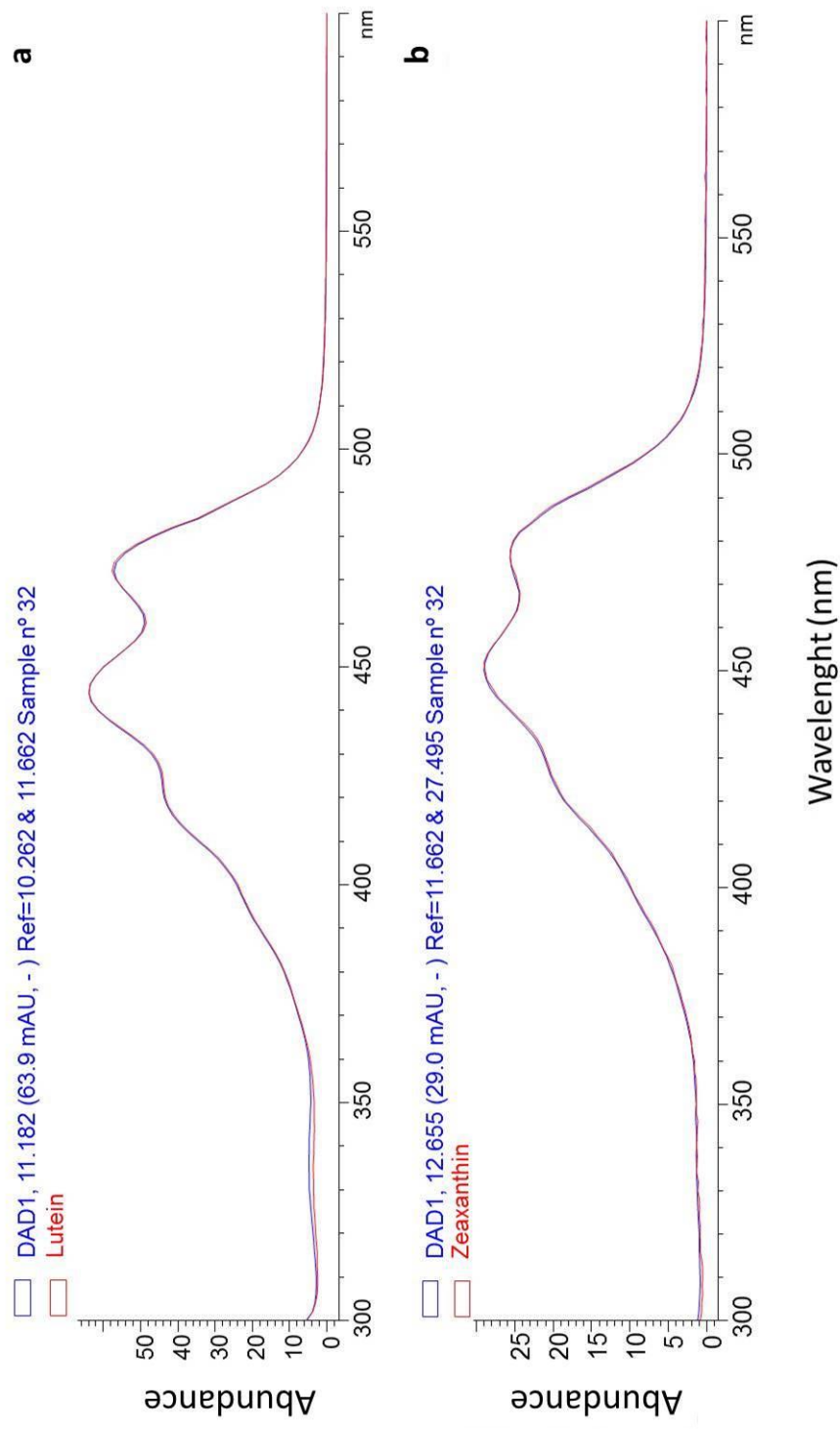
CONCLUSIONS

Our results are consistent with the hypothesis that organic contaminants disrupt carotenoid-based coloration, and may thereby affect communication processes in birds and other vertebrates that rely on these colored traits (McCarty & Secord 2000; Bortolotti et al. 2003). Black Harrier nestlings feeding on more birds had higher levels of circulating carotenoids (which have important health-related physiological functions), but were also more exposed to DDT-contamination. In turn, and paradoxically, DDT-exposed nestlings appeared more carotenoid-limited as they circulated relatively lower carotenoid levels, and developed less orange carotenoid-based integuments. Therefore, the potential benefits of eating carotenoid-rich prey such as birds seemed to be compromised when a nestling was simultaneously exposed to DDTs. An influence of OCs on the expression of carotenoid-based traits may have further implications given their communication roles. Further research is needed to better understand the mechanisms linking OC exposure and carotenoid-based coloration, as well as the broader implications for social communication.

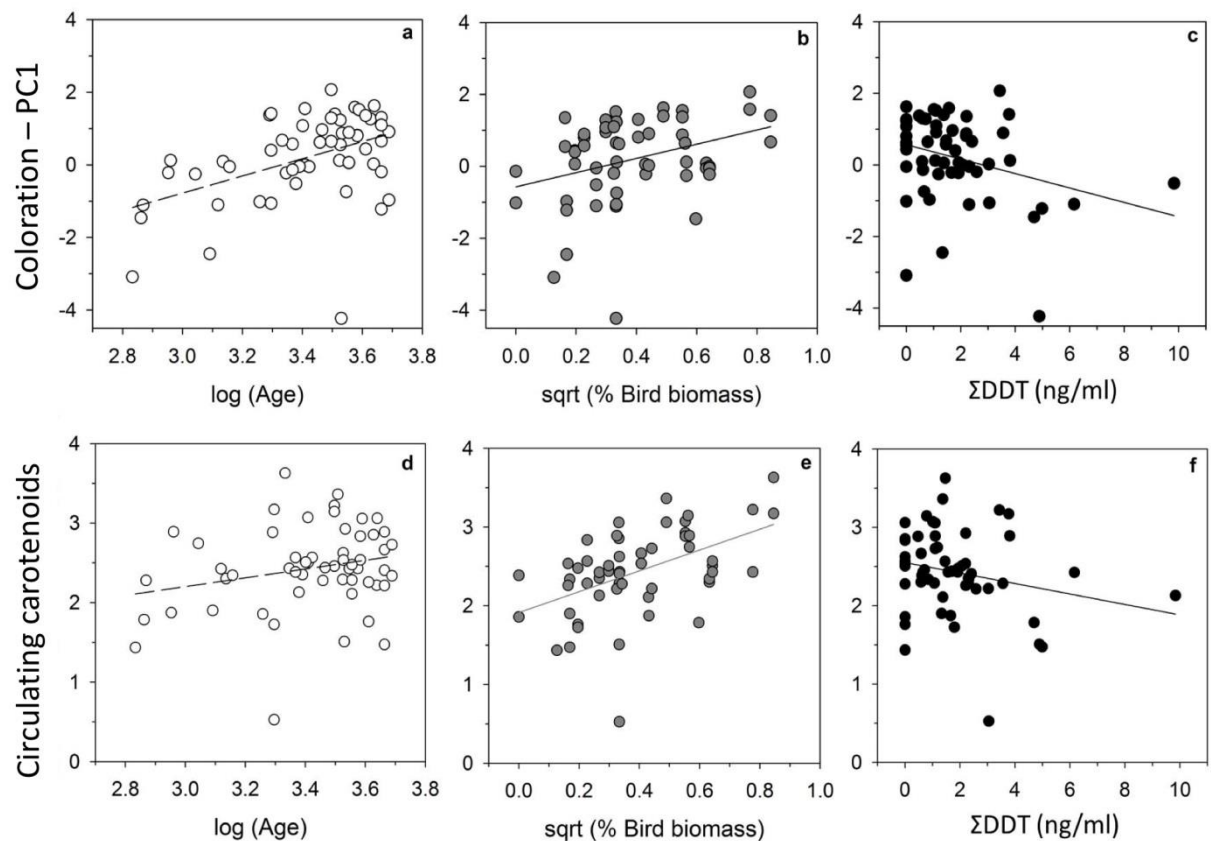
Appendix G: Chromatograph of the carotenoids extracted from the plasma of nestling Black Harriers (n= 101). Carotenoids were identified and quantified using High Performance Liquid Chromatography (HPLC) coupled with a photodiode detector. Lutein and zeaxanthin were the two main carotenoids identified in the samples. The blue solid line represents the Standard used for the carotenoids lutein and zeaxanthin, while the red solid line represents a Black Harrier nestling sample (number 32).



Appendix H: Representation of the lutein (**a**) and the zeaxanthin (**b**) chromatographs: red lines correspond to the Standard, while blue lines are the plasma samples of Black Harrier nestlings. Note the tight overlap between the Black Harrier sample and the lutein and zeaxanthin Standards.



Appendix I: Relationships between coloration-PC1 (i.e. Hue (orangeness)-saturation) or circulating carotenoid levels (in nmol/mL, log transformed) and: nestling age (log transformed) (**a & d**), dietary composition (percentage of bird biomass, square-root transformed) (**b & e**), Σ DDT levels (**c & f**).



General Synthesis



Throughout my PhD research, I have attempted to develop an overall comprehension of how various environmental factors may affect the breeding and health condition of the Endangered Black Harrier, at both the population and the individual level. Conducting a multifaceted study, I have investigated and identified various parameters that could be limiting the population size of this wild ground-nesting bird of prey, and to some extent, may explain its scarcity in South Africa. The outputs of this PhD have important implications for future effective and sustainable conservation of the Black Harrier, as well as for the conservation of other birds of prey. Below I summarize the most important findings of my research, and bring them together to give a complete picture of the factors influencing Black Harrier ecology and conservation status.

Breeding performance, weather and food availability relationships at the population level

Like other birds of prey from the Southern Hemisphere (Martin et al. 2014; Simmons 2000; Simmons et al. 2005; del Hoyo et al. 1994), the Black Harrier exhibits an extended breeding period, with the onset of laying spread over 8 months, from mid-May to mid-December (Chapter 1), but with a clear seasonal peak of laying between mid-August and the end of September. This may indicate that optimal timing for breeding is limited for this species, despite the overall extended breeding period. I further observed a seasonal decline in all breeding parameters, that was progressive and moderate in coastal regions, but much more pronounced in inland regions, for clutch size and productivity specifically. Early in the season, i.e. until September, both clutch size and productivity were relatively higher inland than along the coast, but this tendency reversed from October onwards. However, neither clutch size nor productivity were, on average, significantly different between regions, indicating that regional differences early and late in the season overall balanced each other out. These results provide evidence for spatial variation in the strength of seasonal declines in breeding performance, something that has only received scarce support previously. I further investigated whether variation in weather conditions, i.e. rainfall and temperature, may influence breeding output. I found that these seasonal declines were concomitant with weather conditions becoming drier and hotter in both regions as the breeding season

progressed, these conditions being achieved more quickly inland than in coastal regions. Independently of the nesting region, rainfall negatively influenced laying date, but positively influenced clutch size and productivity: clutches and productivity were greater, and laying occurred earlier if rainfall was high in autumn-spring and when summers preceding laying were wetter. On the other hand, temperatures were not found to have any influence on the timing of breeding or on any breeding performance parameters. These results were somehow surprising, as regional differences in temperatures were observed: temperatures were higher in coastal than in inland regions until August, while the opposite pattern was found from October onwards. In this context, the regional differences in the breeding parameters were likely to be better explained by factors other than weather conditions only, such as habitat quality and/or food availability, the latter of which may also be, in turn, modulated by weather. Overall, these findings suggest that environmental conditions in inland regions may be more suitable than along the coast, but only for a limited period of about 1.5 months, as conditions there quickly deteriorate later in the season inducing poorer breeding performance. In contrast, the timing of breeding may have less importance for the production of young where environmental conditions are less variable and more predictable (i.e. coastal regions). Given the more benign conditions along the coast, a higher number of Black Harrier pairs breed there, and for a longer period of time (8 months): considering all studied years, the number of breeding events was twice as frequent in coastal regions as inland with an average 22 vs. 11 active nests, respectively. Furthermore, when comparing the overall production of Black Harrier fledglings, i.e. the number of nestlings that successfully fledged, between the coastal and inland regions over the study period 2000-2014, numbers showed that, an average 157.8 vs. 52.9 nestlings successfully fledged in coastal and in inland regions, respectively. Accordingly, this suggests that the overall production of nestlings, at the population level and in an average year, may be as much as three times higher in the coastal regions than inland, although this would need to be confirmed with further analyses accounting for searching effort. All this therefore indicates that coastal breeders add much more to future generations than do those breeding inland, and emphasis once again the importance of the coastal Fynbos for the stability and sustainability of the Black Harrier population in the future.

Food resource is a very well-known limiting factor for animals, known to affect the dynamics of wild populations (Newton 1979, 1998). In my Chapter 2, I confirm that Black Harriers specialise on small mammal (ca. 65 % of total identified prey and 78 % of consumed biomass) and primarily feed on the Four-Striped Mouse *Rhabdomys pumilio*. I also show the importance of birds and reptiles as alternative prey for the species. I found significant regional differences in the diet composition of Black Harriers, with small mammals being more prevalent in the diet of coastal breeders, both numerically and in terms of biomass, while more birds were consumed by breeders nesting inland. Reptiles were consumed in similar proportion in both regions.

Given that Black Harrier are specialist predators of small mammals and that as explained above, they show seasonal declines in productivity, I went on to investigate the spatial and temporal variations in the diet composition of breeding Black Harriers. Food availability, i.e. abundance and/or accessibility of food resources, is particularly important for specialist predators feeding on mobile prey (Preston 1990; Newton 1998; Arroyo & García 2006). Habitat and weather conditions are factors that may reduce the accessibility of prey, through modifying their behaviour or probability of capture, which in turn may create situations of food limitation despite high prey abundance (Elkins 1983; Schlaich et al. 2015; Robinson et al. 2016). Although quantifying and measuring the availability of prey in the environment remains a difficult and challenging task (Rosenberg & Cooper 1990; Smith & Rotenberry 1990), an indirect way to evaluate it is to assess the prey provisioning rates using automated camera recording at nests (Zárybnická et al. 2012; Robinson et al. 2016). In Chapter 3, I showed a clear seasonal change in the diet composition of breeders nesting inland. While along the coast the occurrence of small mammals remained constant throughout the season, inland it gradually shifted to fewer small mammals with an increasing representation of birds and reptiles. Furthermore, the analyses of daytime variations in prey provisioning rates revealed that small mammals were delivered markedly less often during the middle of the day at inland nests, whereas alternative prey were still delivered at the same rate (reptiles) or the midday drop was lower (birds). This supports the idea that, during the warmest hours of the day, small mammals become less accessible for hunting Black Harriers, something that was partly explained by variations in temperatures. Indeed, I found that the occurrence of small mammals in Black Harrier's diet significantly

decreased with increasing temperatures. Although I was unable to directly measure prey abundance in my study areas, these results support the idea that hotter temperatures reduce small mammals' availability for harriers because of a reduced accessibility through reduced small mammals' activity (i.e. modified diurnal rhythms) and/or through reduced small mammals' reproduction leading to lower abundance. Additionally, I also investigated the effects of winter rainfall on the occurrence of different prey types in the Black Harrier's diet. Assessing inter-annual comparisons for the coastal breeding sites revealed that, the occurrence of small mammals in Black Harriers diet increased with the previous winter's rainfall suggesting that, at least in this region, winter rainfall positively influences the abundance of small mammals. However, I did not find an association between winter rainfall and small mammal occurrence in the diet when looking at both regions, possibly because the regional seasonal variation in diet is also driven by temperatures.

Overall, this first part of my research, conducted at the population level, suggests that Black Harriers not nesting in coastal regions can breed optimally only during a shorter window, as the primary prey is less available when temperatures are too high. Furthermore, the seasonal decline in the occurrence of small mammals in the diet of inland harriers found in Chapter 3, also coincided with a steeper decline in breeding performance there (Chapter 1). This suggests that the lower prey availability of small mammals in the inland regions, later in the season, is probably limiting reproduction there, forcing breeders to consume harder to catch and/or less optimal prey such as birds or reptiles. Future studies should investigate the apparent narrow link between the diet composition, prey availability, and breeding performance at the individual level, and how this varies in space and time, in order to confirm this hypothesis.

Diet and habitat relationships with contaminant levels and health of Black Harriers, at the individual level

In the next chapters (Chapters 4 and 5), I used an eco-toxicological and eco-physiological approach to assess environmental contamination and its potential effects on wild individuals. It is broadly reported in the literature that persistent organic pollutants, such as polychlorinated biphenyls (PCBs) or organochlorine pesticides (i.e. DDTs and its metabolites)

are extremely toxic to wildlife, causing, among other implications, adverse effects on a number of biomarkers of health of several bird species (Naso et al. 2003; Bowerman et al. 2003; Bourgeon et al. 2012; Gómez-Ramírez et al. 2014 Ortiz-Santaliestra et al. 2015). In Chapter 4, my goal was to assess the presence of organochlorine compounds (OCs) in the plasma of free-living individuals, and to determine potential associations between OC levels, habitat types, diet composition and indicators of physiological condition. I found that 79 % and 84 % of sampled Black Harrier individuals presented levels of, respectively, Σ PCB and Σ DDT (p,p'-DDT + p,p'-DDE). Nestlings presented higher levels of Σ PCB and p,p'-DDT than adult Black Harriers, which in turn presented higher levels of p,p'-DDE than nestlings. As the accumulation of OCs in bird tissues occurs over time (i.e. when intake rate exceeds excretion rate; Kenntner et al. 2003; Goutner et al. 2011), it is generally expected that adults would present higher OC levels than nestlings (Goutner et al. 2011; Ortiz-Santaliestra et al. 2015). My results were in this sense unexpected and worrying as they point towards a recent and local acquisition of PCBs and p,p'-DDT by nestlings on Black Harrier breeding grounds.

I was able to show a positive association between the levels of PCBs in Black Harrier blood and the density of electric transformers located within a breeder's territory, i.e. "Transformer Density Index", which took into account both number and power of those transformers. Those nestlings and adults with the highest levels of blood Σ PCB were also the ones that encountered the greater kVA-rating per km² around their nest, in an area equivalent to the size of the foraging grounds. To the best of my knowledge, this is the first time that such an association has been found in a wild animal, and in the latter context, this index may represent a useful tool to assess the exposure of PCBs and the potential contamination to terrestrial wildlife in general. I further show the key role played by wetlands as a source of contamination by OCs in south-western South Africa: for both nestlings and adults, the p,p'-DDE levels increased with higher proportion of wetlands within Black Harriers territory. This may be the result of former spraying of DDT in the region before it was banned in South Africa in the early 1980s, or its current legal use as part of the malaria control program in malaria risk areas (Wells & Leonard 2006). In the former case, the DDT would have bio-degraded into DDE and bio-accumulated and bio-magnified in local sediments (Hoffman et al. 2003). In the latter case, this contamination may also be due

to the volatilization and condensation properties of DDTs, that migrate from warm source/usage areas, where DDT is sprayed against malaria, to colder regions via long range atmospheric transport (Hoffman et al. 2003; Meijer et al. 2003; Ryan et al. 2012), before its bio-accumulation and bio-magnification in the sediments. Additionally, it may also be indicative of a current illegal use of this pesticide against mosquitoes outside the malaria-risk areas, despite its strict prohibition. This would also further explain the higher levels of p,p'-DDT detected in nestlings, suggesting a recent and local contamination in Black Harrier breeding grounds. Two other possible pathways of contamination are 1) the use of substitutes of DDT that are still legally used in South Africa, such as the dicofol, which is known to often contain p,p'-DDT (Clark 1990; Quinn et al. 2011); or 2) by a maternal-transfer of the p,p'-DDT, and other OCs, to the egg and the embryo, as observed in other raptor species (Bustnes et al. 2008). This would also explain why Black Harrier adult females had lower levels of OCs in their blood than adult males: females may excrete some of these OCs at egg formation, while males continuously bio-accumulate them until they biologically degrade in their bodies (Schnellmann et al. 1985; Hoffman et al. 2003). Furthermore and interestingly, I also found that consuming more birds increased the likelihood of Black Harriers carrying a p,p'-DDE burden. Indeed, those nestlings and adults that had the highest levels of p,p'-DDE, were also the ones that consumed higher proportion of bird biomass. In other words, those individuals that fed on more birds, and especially on farmland birds such as the Common Quail *Coturnix coturnix* (i.e. inland breeders), are more vulnerable and more likely to be exposed to DDT than breeders nesting at the coast, as the latter ones generally feed on more small mammals (Chapters 2 and 3). Remarkably, I found no evidence for protected areas reducing the risk of Black Harriers being contaminated by DDTs or PCBs, suggesting that protected areas may be free of certain disturbances, but not less polluted.

Finally, although detected levels were relatively low, they were within the range or slightly higher than those found in recent northern-hemisphere studies of other raptors (e.g. Sonne et al., 2012; Bustnes et al., 2013; Eulaers et al., 2014; Gómez-Ramírez et al. 2014; Ortiz-Santaliestra et al., 2015), for which adverse physiological effects have also been reported (e.g. Sonne et al., 2012; Ortiz-Santaliestra et al., 2015). Here, I found that PCB contaminated individuals were potentially physiologically stressed and immuno-suppressed, with the Heterophils to Lymphocytes ratio (H:L ratio) increasing with Σ PCB levels, as also

found in other studies (Bustnes et al. 2004). On another hand, p,p'-DDT contaminated individuals had greater number of White Blood Cells (WBC).

For the last chapter of my PhD (Chapter 5), and following on findings of Chapter 4, I conducted further investigations on the potential effects of OCs on additional variables reflecting the health condition of an individual. In this context, I specifically investigated whether OC levels could affect the circulating carotenoid levels and the carotenoid-based colouration of cere and tarsi of Black Harrier nestlings. I also accounted for the potential effects of other variables such as age, sex, brood-rank, physical condition and dietary composition. Carotenoids are important components in avian physiology, serving important health-related physiological functions (Pérez-Rodríguez 2009; Faivre et al. 2003; Blount et al. 2003), but they are also responsible for the carotenoid-based colouration of ornaments, which in turn are known to play key roles in bird communication and social interactions (Bortolotti et al. 2000; Costantini et al. 2007; Sternalsky et al. 2009). In this context, individuals may be facing a trade-off between allocating more circulating carotenoids for self-maintenance, or towards integument colouration (Pérez-Rodríguez et al. 2013). Furthermore, organic and non-organic contaminants have been shown to disrupt the circulating carotenoids and the colouration of plumages and integuments of some bird species (Bortolotti et al. 2003; Blévin et al. 2014; Vallverdú-Coll et al. 2015, 2016b), which may have broader implications for social communication and individual's overall physiological health condition (Marasco & Costantini 2016).

As expected, I found that a greater availability of carotenoid pigments, i.e. higher levels of circulating carotenoids, was associated with a more yellow-orange and purer colouration (hue-saturation colouration) of integuments in nestling Black Harriers. Furthermore, older nestlings were more colourful and presented higher level of circulating carotenoids than younger ones, but no associations were found with the (i) the body condition, (ii) the brood-rank or (iii) the sex of nestlings. (i) indicates that heavier nestlings were not more colourful, nor did they have more circulating carotenoids than lighter ones, and implies that the levels of circulating carotenoids and the colouration of integuments were primarily dependent on the quality (carotenoid content) of the ingested food rather than its quantity; (ii) and (iii) on the other hand, may indicate that food consumption in

terms of carotenoid intake does not differ among nestlings of different sexes or rank within the brood, as found in other studies (Sternalsky et al. 2009, 2012). Furthermore, I found that nestlings consuming more birds presented higher levels of circulating carotenoids and developed more yellow-orange colouration of their ceres and tarsi than the ones feeding regularly on small mammals. While consuming more birds seems beneficial for nestling Black Harriers as it increases the levels of circulating carotenoids (which have important health-related physiological functions; Lozano 1994), it also increases the likelihood of ingesting DDT, as explained above. In turn, and paradoxically, DDT-exposed nestlings appeared more carotenoid-limited as they circulated relatively lower carotenoid levels, and developed less orange carotenoid-based integuments. Therefore, the potential benefits of eating carotenoid-rich prey such as birds seemed to be compromised when a nestling was simultaneously exposed to DDTs. Additionally, I also found that the circulating carotenoid levels and the orangeness-purity of coloured integuments were unrelated to blood PCB levels, although the brightness of integuments (i.e. lack of pigmentation indicative of whiter, less pigmented integuments) increased with PCB levels. PCBs could affect nestlings through the activation of the cytochrome P450 enzyme system by PCBs, which acts on the cycles of oxidation-reduction and generates free radicals (Schelzinger et al., 1999), which may thereby increase the production of pro-oxidant molecules, and in turn may cause oxidative stress. By increasing oxidative stress, PCBs could also affect carotenoid allocation priorities: carotenoid pigments have antioxidant properties and their allocation to coloured integuments is usually reduced under conditions of increased oxidative stress (e.g. Mougeot et al., 2010; Perez-Rodriguez et al., 2010; Vallverdu-Coll et al., 2015, 2016a). All together, these results are consistent with the hypothesis that OC contaminants, in particular DDT, may disrupt carotenoid-based signaling in exposed nestlings. This probably arises because nestlings have to mobilize more circulating carotenoids for self-maintenance in order to compensate for the adverse effects of DDTs, and would as a result be less able to deposit them into coloured integuments such as cere or tarsi.

Overall, Chapters 4 and 5 contribute new knowledge on the exposure and the transference of organochlorine compounds to wildlife in South Africa, but also add information to the overall understanding on how these OCs may be affecting the physiological condition of individuals, and the expression of carotenoid-based traits. In a

nutshell, adult and nestling Black Harriers are not safe from agro- and industrial- associated chemicals, even within protected areas, where they appear to pick up p,p'-DDE in areas where DDT has been banned since the early 1980s, and get highly contaminated by PCBs due to the presence of electricity transformers within protected areas. To this, the consumption of avian prey contributes increasing contamination risks of Black Harriers by p,p'-DDE, most notably for inland breeders. All this may have important negative consequences for Black Harriers in the long term as OC-contaminated individuals showed lowered immune responses, which in turn may compromise their ability to fight off pathogens or parasites that they may encounter in the wild. Future research is needed to address how these OCs are inducing physiological disruptions in Black Harriers, and to obtain a broader comprehension on the mechanisms linking OC exposure and carotenoid-based colouration, as well as the broader implications for social communication.

GENERAL CONCLUSIONS AND FUTURE RESEARCH

This thesis has investigated Black Harriers at both the individual and population levels, to clarify specific threats to the long-term conservation and survival of this rare raptor. A graphical representation of the main results and conclusions is shown in Figure 24. Overall, my results highlight the importance of the coastal Fynbos for the stability and sustainability of the Black Harrier population in the future. With a milder and more temperate climate, coastal regions (Fynbos biome) seem to be more optimal, allowing Black Harriers to breed successfully over longer periods as environmental conditions there seems to remain more stable within and among years. Conversely, other suitable sites located further inland, in the Karoo biome, seem to be environmentally more unstable and variable and may become drier and hotter quickly in late spring and early summer, offering a shorter window of opportunity for breeding harriers. Thus, even if average productivity per nest is similar in both regions, coastal regions probably contribute to producing more fledglings per km² annually than inland regions. Additionally, these regional differences may be further exacerbated by the recent changes in climate conditions observed within Africa during the last decade (Hulme et al. 2001; Hockey et al. 2011; Kruger & Sekele 2012; Cunningham et al. 2015). Indeed, a shift in rainfall and temperature patterns has occurred in South Africa, and

most notably in the Cape Floral Kingdom and the succulent Karoo, which also coincides with Black Harriers breeding range. In these areas, temperatures are predicted to become warmer and winter-rainfall less frequent, with longer and more frequent droughts (Midgley et al. 2002; Hockey et al. 2011, Cunningham et al. 2015). This, together with the results of Chapter 1, indicates that climate change may influence Black Harrier's breeding phenology and its breeding outputs, all linked with primary prey availability (Chapter 2 and 3). Indeed, these negative changes in weather conditions are likely to further reduce Black Harriers' food resources by limiting the availability, i.e. accessibility and abundance of their primary prey, small mammals. This could result in Black Harriers increasingly consuming alternative prey such as birds, potentially increasing the likelihood of DDT contamination. This switch to alternative prey, may in turn lead to decreased foraging success and as a consequence breeding outputs, as evident in other specialist predator species (e.g. Simmons et al. 1986; Korpimäki 1992; Arroyo & García 2006; Terraube et al. 2012).

In addition to the above, anthropogenic modifications of land use during the last century in South Africa, such as the conversion of the Fynbos vegetation into agriculture or urbanization, has contributed negatively to affect the Black Harrier population (Curtis et al. 2004; Curtis 2005). The destruction of this natural vegetation has dramatically reduced the availability of Black Harrier's breeding habitat, and the continuous increase of urbanization and agricultural lands exacerbates the likelihood of contamination by pollutants (PCBs and DDTs) to Black Harriers, and to wildlife in general. In turn, these OC exposures also induced sub-lethal and physiological negative effects in contaminated individuals. The fact that protected areas did not reduce the risk of Black Harriers being contaminated by OCs is worrying and should create urgency to identify these sources of contamination and implement future efficient conservation measures towards its reduction. Within this context I suggested that the scarcity of Black Harriers may be related to a lack of optimal (un-urbanised, un-fragmented, un-polluted and food-rich) areas for breeding.

Although I have focused my PhD research on breeding populations only, future studies should consider limits imposed in the non-breeding season. Whereas the species has been observed to migrate eastwards in South Africa in summer (see blog of RES: <http://blackharrierspace.blogspot.co.za/2015/01/the-season-for-migration-east.html>,

unpublished data; Taylor 2015), very little is currently known about what Black Harriers do during the non-breeding season (see RES blog). Even less is known about the potential risks and factors that individuals may be facing during that period of the year. The identification of critical areas used by species during their entire annual cycle (including breeding, migration stop-overs and non-breeding) are fundamental components, for effective conservation efforts and efficient wildlife management and planning (Börger et al. 2008; Estrada et al. 2010; Marini et al. 2010; Real et al. 2010; Carvalho et al. 2011). This would help conservation measures for the long-term sustainability of the species in space and time. Conservation measures have already prioritized the protection of the fragile and vulnerable Fynbos vegetation, through the creation of national parks and private reserves. However, a strengthening of the law, reinforcing the protection and conservation of these natural habitats, with the strict prohibition of further habitat destruction, will help reduce further fragmentation. The preservation and protection of natural habitats of good quality (i.e. un-polluted and food-rich) should continue in order to insure the conservation of Black Harriers in the long term. As good predictors of small mammal and avian biodiversity (Jenkins et al. 2013) , the conservation of Black Harriers will have positive spinoffs for the conservation of many other terrestrial species that face similar threats.

Overall, the results of this thesis highlight the importance of multifaceted studies, at both the population and individual levels, when attempting to understand a species' limiting factors. Indeed, the reasons for the scarcity or decline of wild species are unlikely to be related to a single factor, but rather the result of a succession and additive effects of several factors (Gaston 1994). In this context, it seems essential to investigate key parameters known to influence the dynamics of wild animal species, such as the spatial-temporal variations of the breeding phenology and success, and the diet composition and/or food requirement of a species. In my thesis, I have shown the importance of identifying breeding areas and breeding habitat types of good quality in terms of food availability and weather conditions. These, in turn will provide the best conditions for individuals to breed successfully and ultimately to insure the stability and sustainability of those species. Furthermore, the identification of threats, such as contamination by pollutants, which are known to strongly affect wild populations of raptors, should also become a priority when studying wild animal populations. Investigating how these threats may affect the physical

and physiological health of individuals is a further step towards a more global comprehension of the system where those species evolve. All this is even more important when dealing with threatened species of small population size. Indeed, those are usually more exposed to the loss of genetic diversity and can suffer from a diminished capacity to respond to environmental changes, such as pollutant contamination or climate change (e.g. increase of temperatures and decrease in rainfall, which may also affect primary prey abundance), and hence, a reduced adaptive potential. In other words, it seems essential to understand how several abiotic and biotic parameters may affect wild populations, and to combine this information to better understand the potential factors playing a role in the scarcity of a species. This will allow designing efficient and sustainable conservation measures for the latter ones, but also for the conservation of many other species that may face similar threats all around the world (Sutherland et al. 2004).

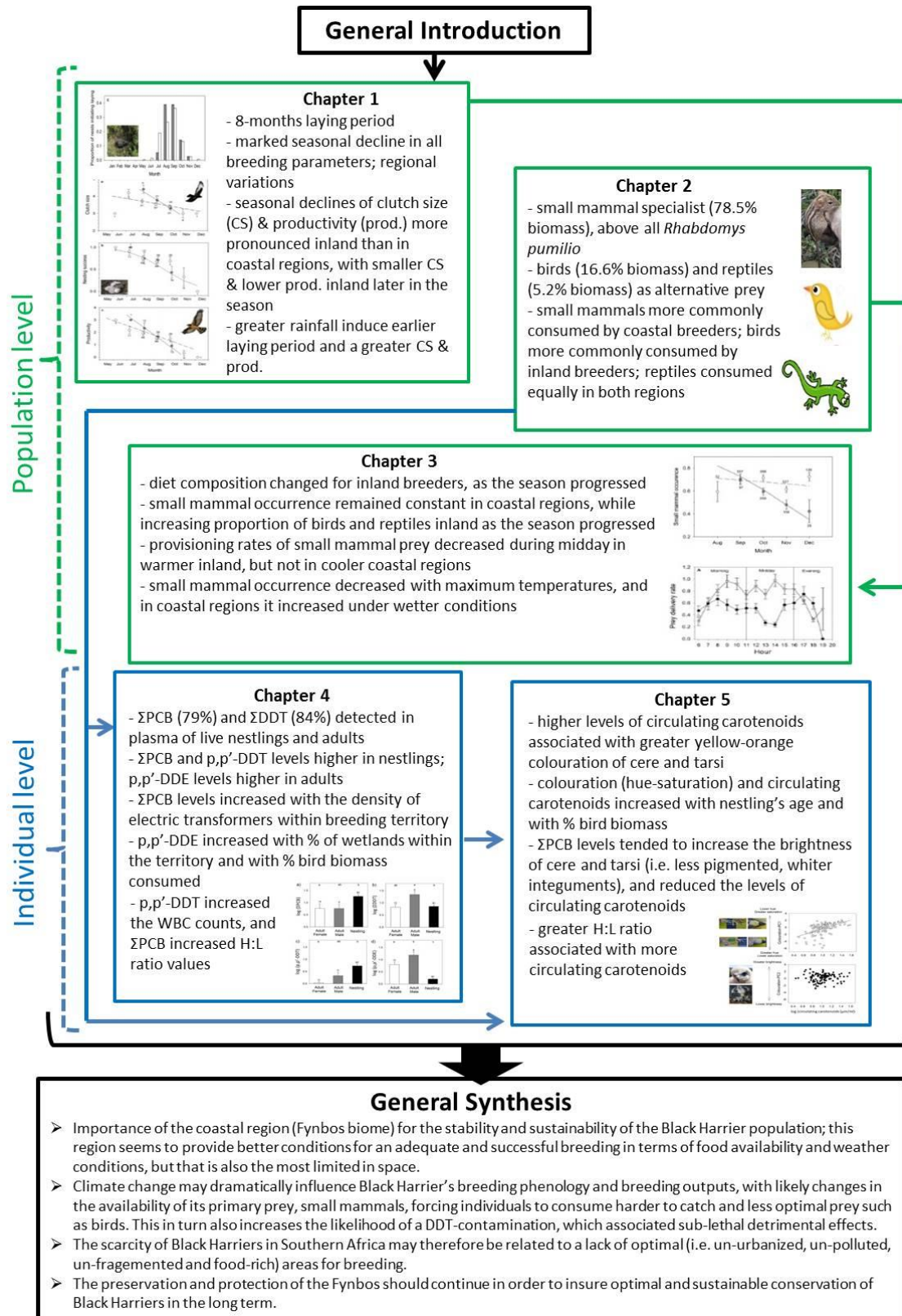


Figure 24. Schematic representation of the main findings of each chapter of my PhD investigation, at both the population (green colour chapters) and at the individual level (blue colour chapters), and how they relate to each other to form the general synthesis of my research.

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